Biochemistry (Moscow), Vol. 68, No. 2, 2003, pp. 247-251. Translated from Biokhimiya, Vol. 68, No. 2, 2003, pp. 299-303.

HYPOTHESIS

Induced Absorption Band of Holotransketolase and Its Interpretation

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Received March 19, 2002

Abstract—It has long been known that formation of a catalytically active holotransketolase from the apoenzyme and thiamine diphosphate (ThDP) is accompanied by appearance, in both the absorption and CD spectra, of a new band. Binding and subsequent conversion of transketolase substrates bring about changes in the intensity of this band. The observation of these changes allows the investigator to monitor the coenzyme-to-apoenzyme binding and the conversion of the substrates during the transketolase reaction and thus to kinetically characterize its individual steps. As regards the new absorption band induced by ThDP binding, its nature, until recently, remained unknown. The reason for its appearance was considered to be either the formation of a charge transfer complex between ThDP and tryptophan (phenylalanine) residue or stacking interaction between the residues of aromatic amino acids. They are thought to be brought together as a result of conformational changes of the apoenzyme during its interaction with the coenzyme. However none of these hypotheses had been substantiated experimentally. According to our hypothesis, the induced absorption band is that of the imino form of ThDP resulting from three contributing features of the ThDP binding site of transketolase: the relative hydrophobicity of this site, hydrogen bonding of the N1′-atom of the ThDP aminopyrimidine ring to Glu418, and base stacking interactions between the aminopyrimidine ring of ThDP and Phe445.

Key words: thiamine diphosphate, transketolase, new absorption band, circular dichroism, absorption spectrum

Transketolase (EC 2.2.1.1) from the yeast *Saccharomyces cerevisiae*, a ThDP-dependent enzyme, is a transferase that catalyzes the reversible transfer of a two carbon residue from keto- to aldo-substrates [1]. The enzyme has been studied in considerable detail. A great deal of X-ray data have been collected on the 3D structures of the apo- and holoenzyme [2-4] as well as on various transketolase complexes with ThDP analogs [5, 6] and a holotransketolase complex with the acceptor substrate [7]. The combination of site-directed mutagenesis, X-ray structural studies, the reaction kinetics, and CD spectroscopy data have been used to characterize the amino acid residues involved in the interactions with ThDP, in substrate channel formation, and in catalysis [4, 8-12].

Transketolase is composed of two identical subunits and has two active centers [3, 13, 14]. ThDP is bound at the interface between the subunits and interacts with residues from both subunits [3]. Comparison of the 3D structures of some thiamine diphosphate enzymes has shown that the ThDP molecule has so-called "V"-conformation which provides a direct contact between the amino group of the pyrimidine ring and the C2–H bond of the thiazolium ring [15]. Thus both subunits are involved in active center formation, and the dimer can be considered as a catalytically competent unit.

The aminopyrimidine ring of ThDP is located in a hydrophobic pocket formed by aromatic side chains, Phe442, Phe445, and Tyr448 (Fig. 1). The side chain of Phe445 is in stacking interaction with the aminopyrimidine ring. Besides, the aminopyrimidine ring forms several hydrogen bonds, the most significant of which is one between the N1′-atom of the aminopyrimidine ring and the side chain of Glu418 [3, 4]. Analogous interaction is observed with other ThDP-dependent enzymes [16-18] and plays an important role in the mechanism of thiamine catalysis.

The interaction of apotransketolase with ThDP is accompanied by the appearance of a new band in the absorption spectrum (range 285-370 nm, Fig. 2a [19]) and in the CD spectrum (range 300-380 nm, Fig. 2b, curve *2* [20]), which was lacking in the initial components [19-22]. There is a clear-cut correlation between the quantity of ThDP bound to the apoenzyme and the catalytic activity [23]. This optical effect is used in experi-

Abbreviation: ThDP) thiamine diphosphate.

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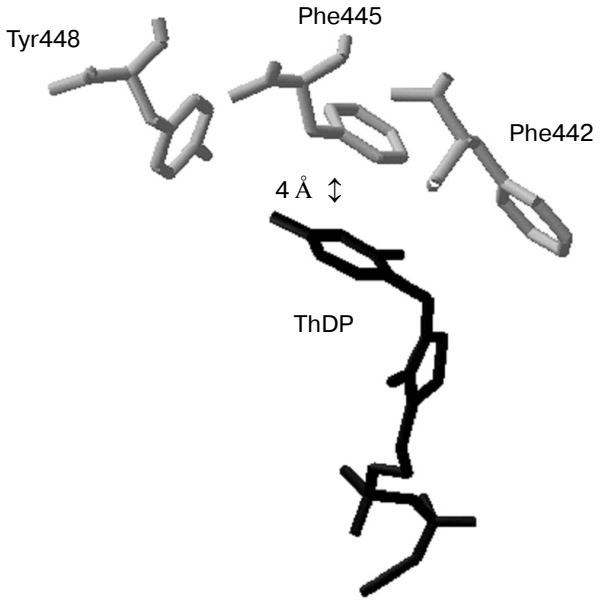


Fig. 1. The hydrophobic "pocket" of the holotransketolase active center. Atomic coordinates are taken from Protein Data Bank (1 trk).

ments to investigate the process of ThDP-to-apotransketolase binding [23, 24]. The interaction of the substrates with holotransketolase and their subsequent conversion is accompanied by significant changes in the intensity of the new absorption band [19, 21], and these changes are widely used for characterization of individual steps of the transketolase reaction [20, 25-27].

Thus far, the nature of the new absorption band of holotransketolase has not yet been identified. Earlier it was suggested that emergence of the new absorption band of holotransketolase is the result of the charge transfer complex formation between ThDP and a tryptophan residue in the transketolase active center [19]. However, according to X-ray data, the tryptophan residue closest to ThDP is 19 Å away from it. (Data are taken from Protein Data Bank (1 trk)).

When the 3D structure of holotransketolase had been solved, the suggested tryptophan function was attributed to the Phe445 residue which is in stacking interaction with the aminopyrimidine ring of ThDP [4, 8] (Fig. 1). To verify this suggestion, Phe445 was replaced by Ile. This replacement maintains the hydrophobic environment around the pyrimidine ring, but the aromatic ring disappears. The CD spectrum of mutant F445I has shown that this replacement does not lead to the disappearance of the induced absorption band, but is accompanied by a 5-fold decrease in its intensity [28].

Another explanation was then offered. As known, the ThDP binding to the transketolase active center, followed by conformational changes in the protein molecule, leads to stabilization of two loops, which are flexible in apotransketolase and well ordered in holotransketolase [2]. As a result, the Trp391 residue from one loop and the Tyr370 residue from the other are brought closer together while their aromatic rings become parallel to each other. Based on this finding, it was suggested that the new, ThDP-induced, absorption band, is caused by stacking interaction between the aromatic rings of the two residues [28]. To ascertain if this is indeed the case, Trp391 was replaced by Phe. However the CD spectrum of the mutant holotransketolase thus obtained proved very similar to that of wild type holotransketolase (Wikner, C., and Meshalkina, L., unpublished data).

Thus none of the suggestions proposed to elucidate the nature of the induced absorption band whose appearance accompanies the ThDP–apotransketolase interaction and the catalytically active holoenzyme formation appears plausible.

In this paper, a new hypothesis for the appearance of the induced absorption band of holotransketolase is proposed, according to which this phenomenon is caused by changed

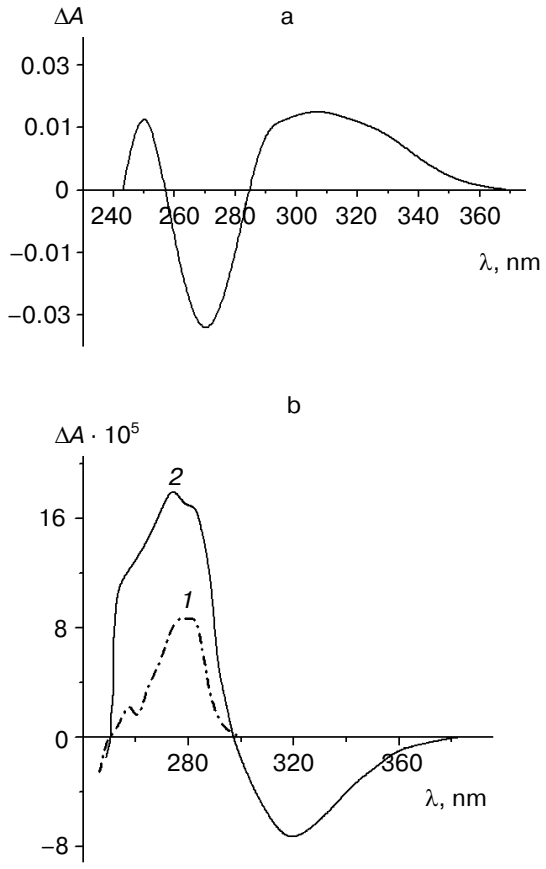


Fig. 2. Absorption spectrum of holotransketolase with respect to apotransketolase and ThDP (a) [19] and CD-spectra of apotransketolase (*1*) and holotransketolase (*2*) (b) [20].

optical properties of ThDP following its incorporation into the hydrophobic "pocket" of the enzyme active center. Given below are the data in support of this hypothesis.

When ThDP is placed into medium less polar than water, a band appears in its spectrum that is absent when the coenzyme is in an aqueous environment (Fig. 3a, curve *1*). An analogous band in the near UV region of the spectrum was detected when thiamine was monitored in dioxane (Fig. 3b). It should be noted that in both cases the new absorption band appears in the same region of the spectrum as the induced absorption band of holotransketolase (Fig. 2a). Moreover, the spectrum in Fig. 3b (thiamine in dioxane) [29] is analogous to the one in Fig. 2a (holotransketolase) not only in the induced absorption band region but throughout the investigated wavelength interval.

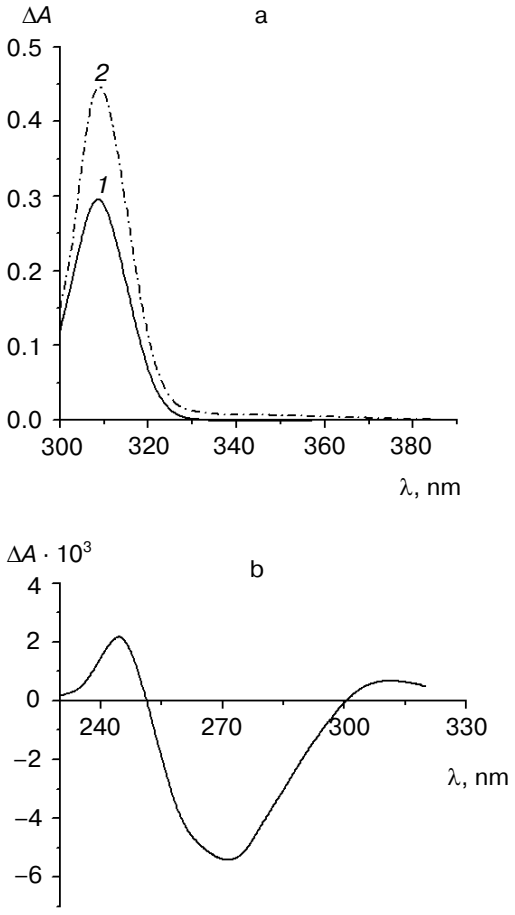
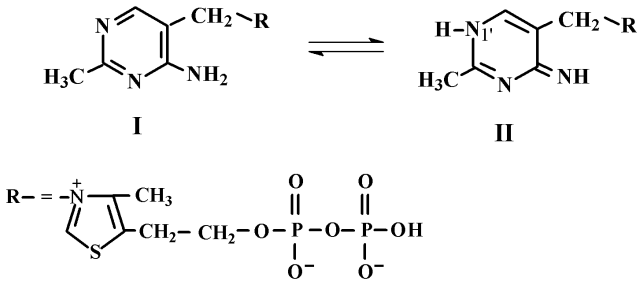


Fig. 3. Absorption spectra of ThDP (2.5 mM) in ethanol solution (50%) in the absence (curve *1*) and presence (curve *2*) of phenylalanine (2.5 mM) with respect to ThDP in water and ThDP + phenylalanine in water, respectively (a), and absorption spectrum of thiamine in dioxane against thiamine in water (b) [29].

In the presence of phenylalanine, the intensity of the ThDP absorption band (300-320 nm) in the ethanol solution is increased (Fig. 3a, curve *2*), consistent with the data obtained with mutant transketolase: the replacement of Phe445, located 4 Å away from the aminopyrimidine ring of ThDP (Fig. 1), for Ile leads to a significant decrease in the intensity of the induced absorption band [28].

Bearing in mind the hydrophobicity of the transketolase active center, the data on the induced absorption band of holotransketolase were interpreted as resulting from changed optical properties of the coenzyme following its incorporation into the hydrophobic pocket of the active center. Apparently the intensity of the induced absorption band increases at the cost of the interaction between the ThDP aminopyrimidine ring and the Phe445 residue.

As known, there occurs interconversion of tautomeric forms of the ThDP aminopyrimidine ring during catalysis (Scheme) at the cost of the hydrogen bond formation between carboxyl group of Glu418 and the N1′-atom of the ThDP aminopyrimidine ring. Fast and easily reversible interconversion between these two forms of the ThDP aminopyrimidine ring is a necessary requirement for the enzymatic catalysis [30].



Scheme

Tautomeric forms of ThDP

In aqueous solution ThDP largely occurs as the amino form (I in Scheme), having no absorption bands in the near UV region [31]. Hydrophobic environment promotes the formation of the imino form [32] (II in Scheme), which (in the holoenzyme) may be stabilized through its interaction with the active center amino acid residues—in particular, with the glutamic acid residue at the cost of its interaction with the N1′-atom of the aminopyrimidine ring [33, 34]. In the case of transketolase this residue is Glu418 [4, 8]. Indeed, the interaction of transketolase mutant (where the Glu418 residue is replaced by Glu or Ala) with ThDP was not accompanied by the appearance of a new absorption band with maximum at 320 nm in CD spectrum [8]—just opposite to the case of the coenzyme interaction with the wild type enzyme.

Together, the data presented herein allow us to formulate a general concept of the nature of the induced optical band of transketolase-ThDP and the reason for its appearance.

During the interaction of ThDP with apotransketolase the aminopyrimidine ring of the coenzyme incorporates into the hydrophobic "pocket" of the enzyme active center, after which its 4′-aminotautomeric form of the aminopyrimidine ring converts to the N1′H-iminotautomeric form stabilized through the interaction with the Glu418 and Phe445 residues.

The investigation of a variety of catalytically inactive ThDP analogs has shown that their interaction with transketolase is either not accompanied by the appearance of the induced optical band or the character and intensity of optical changes differ from those occurring in the native holoenzyme [35]. There is only one exception: N3′-pyridyl-ThDP, in which N1′-atom was replaced for carbon. The optical properties of the holotransketolase bound to this analog do not differ from those of the native holoenzyme [36]—the α-aminopyridine ring of this analog like the aminopyrimidine ring of ThDP possess the ability to convert into the imino form (N3′H-iminotautomeric form) [37].

The fact that ThDP after the replacement of N1′ for carbon (N3′-pyridyl-ThDP) has lost its catalytic activity when incorporated into the holoenzyme, can be explained by the lacking of the N1′-atom, which is capable of forming a hydrogen bond with Glu418 residue—a necessary requirement for catalysis.

Generally recognized for all thiamine diphosphate enzymes is the immediate participation of the N1′H-iminotautomeric form of ThDP in the act of catalysis. In the case of transketolase this form arises already at the step of the coenzyme binding to the apoprotein, prior to the act of catalysis. This could concern also some other thiamine diphosphate enzymes; in particular, it has been shown that the holopyruvate dehydrogenase components of the pyruvate dehydrogenase complexes from pigeon breast muscle [38] and bovine kidney [39] possess the induced absorption band upon thiamine diphosphate binding in the same spectral region as holotransketolase. With other thiamine diphosphate enzymes there may be another situation: namely, ThDP could bind with apoprotein in the amino form and develop imino form only during the catalysis. As for the catalysis itself, its mechanism remains unchanged an all cases.

This work was supported by the Russian Foundation for Basic Research (grant No. 99-04-49121) and program of the Russian Federation Ministery of Industry and Science "International Projects". We would like to thank Prof. L. S. Yaguzhinsky and Dr. V. M. Demyanovich for helpful discussions.

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