Cooperativity and flexibility of active sites in homodimeric transketolase

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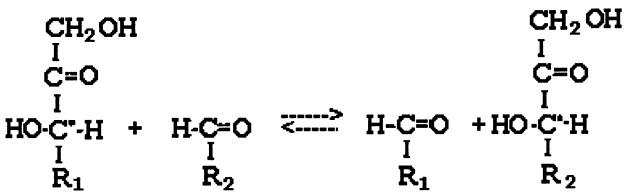
Abstract Here we summarize evidence for non-equivalence of two structurally similar active sites in transketolase and other thiamine-dependent enzymes. This non-equivalence takes place when the enzymes interact with various ligands (inhibitors, cations, coenzyme and substrates). Data on different strains in the structure of the holotransketolase subunits are also given. The above results are discussed within the framework of a concept of permanent alternative site oscillation of the transketolase molecule in the presence and in the absence of substrate as a manifestation of a 'flip-flop' mechanism.

Key words: Transketolase; Flip-flop mechanism; Alternative site reactivity; Half-of-the-sites reactivity; Active site flexibility

Transketolase is a dimeric enzyme with two symmetrical active sites, which are located in the interface of identical subunits and are formed only in the presence of the coenzyme thiamine diphosphate (ThDP) [1]. Transketolase transfers the two-carbon fragment (glycolaldehyde residue) from ketose (donor substrate) to aldose (acceptor substrate):



The transketolase reaction is reversible, except when hydroxypyruvate is used as a donor substrate. In this case, transketolase, like other thiamine-dependent diphosphate enzymes (pyruvate decarboxylase and 2-oxoacid dehydrogenases), decarboxylates the ketose substrate.

There is no direct evidence that transketolase acts in a 'flip-flop' mode (counterdirected or alternative work of active sites during catalysis). However, there are some indirect data that support this notion.

It is known that the active sites of transketolase (TK) are kinetically non-equivalent. They have different (10-fold or more) affinities to cations and the coenzyme [2–6]. Chemical modification of TK often exhibits biphasic inactivation. The biphasity was explained by different rates of active site inactivation [7,8]. On the other hand, the active sites and the whole subunits of TK have the same structure [1,9]. Besides, the catalytic activities of both sites are known to be the same and their optical characteristics change in the same way when ligands are bound [2,10]. Thus, the difference between active sites in TK only shows up on functional level.

The above conclusion is also supported by data on some other thiamine-dependent enzymes. For example, in pyruvate decarboxylase active sites also have different affinity to ThDP [3], although X-ray crystallographic analysis has shown their structural identity [11]. The 3D-structure of pyruvate dehydrogenase is yet unknown, but the primary structures of their subunits are identical [12]. Half-of-the-sites reactivity of pigeon muscle pyruvate dehydrogenase was shown both by an optical method [13] and by an inhibition analysis [14,15].

Direct evidence for the non-equivalence of the TK active sites with respect to substrates was obtained in the studies of ligand effects on the rate of enzyme inactivation by tetranitromethane [16]. The donor substrate (ketose) turned out to cause a sharp change in holoTK inactivation: its rate increased but the inactivation stopped at ~50% residual activity (compare curves 2 and 3, Fig. 1). The inactivation kinetics of semi-holoTK holosites[1] was also changed in the presence of ketose (compare curves 1 and 2). The semilog plot remains linear, however, and its slope is equal to that for the rapid phase of holoTK inactivation in the presence of ketose (compare curves 3 and 1). Therefore, the biphasity of curve 3 reflects rapid inactivation of one site of holoTK and protection of the other. The reactive site clearly contains substrate, as otherwise one could not observe the substrate effect on the inactivation kinetics of semi-holoTK. It is unknown whether the second ('protected') site contains a substrate. Taking into account that the holodimer is twice as active as semi-holoTK [7,17,18], it seems unlikely that one site does not bind the substrate until the other gets free. Therefore, we believe that both sites contain substrate, but in different states (split and non-split), and work simultaneously, but counterdirectly: when ketose is split in one site, it is formed in the other and vice versa.

The results of the experiment in which holoenzyme was incubated with radioactive ketose ([I-^{14}C]fructose 6-phosphate) [19] confirms the heterogeneity of TK in the presence of ketose. One half of bound radioactivity has proved to belong to active glycolaldehyde (an intermediate of the transketolase reaction), the other basically belongs to ketose.

Thus, the above data obtained by different methods indicate that during ketose binding with TK active sites it is only split in one of them.

This conclusion also explains the difference in holoTK spectral responses to the addition of reversibly (fructose 6-phosphate (F6P)) and irreversibly (hydroxypyruvate (HP)) split substrates (Fig. 2) [20,21]. The changes in circular dichroism

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Abbreviations: TK, transketolase; ThDP, thiamine diphosphate; F6P, fructose 6-phosphate; HP, hydroxypyruvate

[1] The holosite (aposite) is here the site which contains (does not contain) ThDP. Semi-holoTK only contains ThDP in one of the two active sites [7]. Activity of semi-holoTK was measured without addition of ThDP to detect the activity of the holosites only.

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spectrum of the holoTK in the region of 300–340 nm caused by ketose addition are due to formation of the intermediate of the transketolase reaction, dihydroxyethyl ThDP, bound to apoprotein [22]. But the amplitude of the holoTK spectral response to the addition of F6P (the difference between curves 1 and 2 at 300–340 nm) is almost twice as low as the response to the addition of HP (the difference between curves 1 and 3). This fact will be understood, if one accepts that the irreversibly split substrate (HP) undergoes catalytic transformation (decarboxylation) in both active sites simultaneously while the reversibly split substrate (F6P) is converted in only one site. The latter is possible if the value of K_d for aldose (the leaving component of the reversibly split substrate) for one of the TK active sites is much less than the value of the integral K_m for aldose, such that the reversibly split-off aldose cannot leave two sites simultaneously.

That TK active sites interchange counterdirectly and permanently their alternative states is also confirmed by modeling the interaction between the enzyme and ThDP [23,24]. The different affinity of TK active sites to ThDP has been proven to be caused by the difference between the rate constants of the backward reaction of the slow (conformational, final) stage of coenzyme binding. In other words, the difference between the sites appears only after ThDP binds to the sites in the final stage. Such a difference is caused by destabilization of the ThDP complex in one of the two identical sites. This destabilization should take place with the same probability in either site, and its reversibility leads to a permanent oscillation of the TK molecule between two asymmetric states.

The reason for TK oscillation could be an asymmetrical strain in the structures of its active sites. This assumption is supported by the crystallographic data. According to the temperature factor distribution, one subunit is 'hotter' than the other; the coordinates of its residues are more 'noisy' and are defined less accurately [9]. A visual geometrical manifestation of the asymmetry strain in TK structure is presented by the position of the Trp391/Tyr370 couple and its environment in different subunits of the enzyme (Fig. 3). The hydrophobic

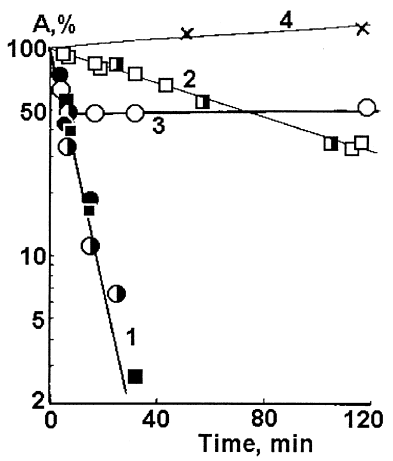

Fig. 1. The influence of ligands on the kinetics of TK inactivation by tetranitromethane [16]. 1: ApoTK (■), apoTK in the presence of ketose-substrate (F6P) (●), and semi-holoTK in the presence of F6P (◑); 2: holoTK (□), and semi-holoTK (◨); activity of semi-holoTK both in the presence and in the absence of ketose was measured without ThDP addition to detect the activity of the ThDP-containing sites only; 3: holoTK in the presence of F6P (○); 4: control in the absence of the inhibitor (×).

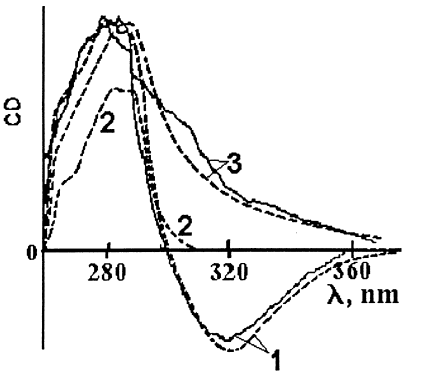

Fig. 2. CD spectra of holoTK (1), holoTK+F6P (2), holoTK+HP (3) [20,21].

(possibly stacking [21]) interaction between these residues is thought to be necessary to stabilize the flexible ThDP-binding loops of the active site [21]. Fig. 3 makes clear that Tyr370 rings and some other side chains are oriented differently in two subunits. It is obvious that the differences shown are the result of asymmetrical strain within crystalline holoTK. In solution, it could result in a one-by-one destabilization of the holosites.

Another puzzle which can be easily explained in terms of the 'flip-flop' mechanism, is slow (tens of minutes [3]) dissociation of bound coenzyme and its fast (less than 20 s [25,26]) exchange with the medium coenzyme. Apparently, because of the one-by-one destabilization of holosites, the ThDP molecule bound in the transiently destabilized site may be shifted in a way allowing binding of a second ThDP molecule from the medium. When this site gets back to its original state, a severe competition between the 'new' and the 'old' ThDP arises, explaining the rapid exchange between the bound and the medium ThDP. Therefore, the rate of TK oscillation is likely to coincide with the rate of the ThDP exchange. It was also shown that the rate of the exchange between bound ThDP and dihydroxyethyl-ThDP[2] of the medium coincides with the rate of catalysis [27].

Of course, each of the above considered phenomena can be explained not only by the permanent counterdirected oscillation of enzyme structure. But only the mechanism of one-by-one counterdirected work of the active sites agrees with all data on the structure and the catalytic behavior of TK. It should be noted that the mechanism described above is not exactly the 'flip-flop' mechanism of Lazdunski [28–30]. His 'flip-flop' mechanism implies such a strong interdependence of the sites that they are unable to function without each other, i.e. if one site is inactive, so is the other. By contrast, active sites can function independently in TK, as shown for the cases when one of the sites is inactive [7,16–18] or when the connection between the sites is broken (the biphasity of the inactivation kinetics shown in Fig. 1 disappears after a long storage – the sites begin to work independently). Taking into consideration the data on the functional flexibility of the TK molecule [31,32], we believe that the absence of the influence of one active site upon another in the above mentioned cases does not contradict the idea about counterdirected work of active sites in native TK.

[2] The intermediate of the TK-reaction.

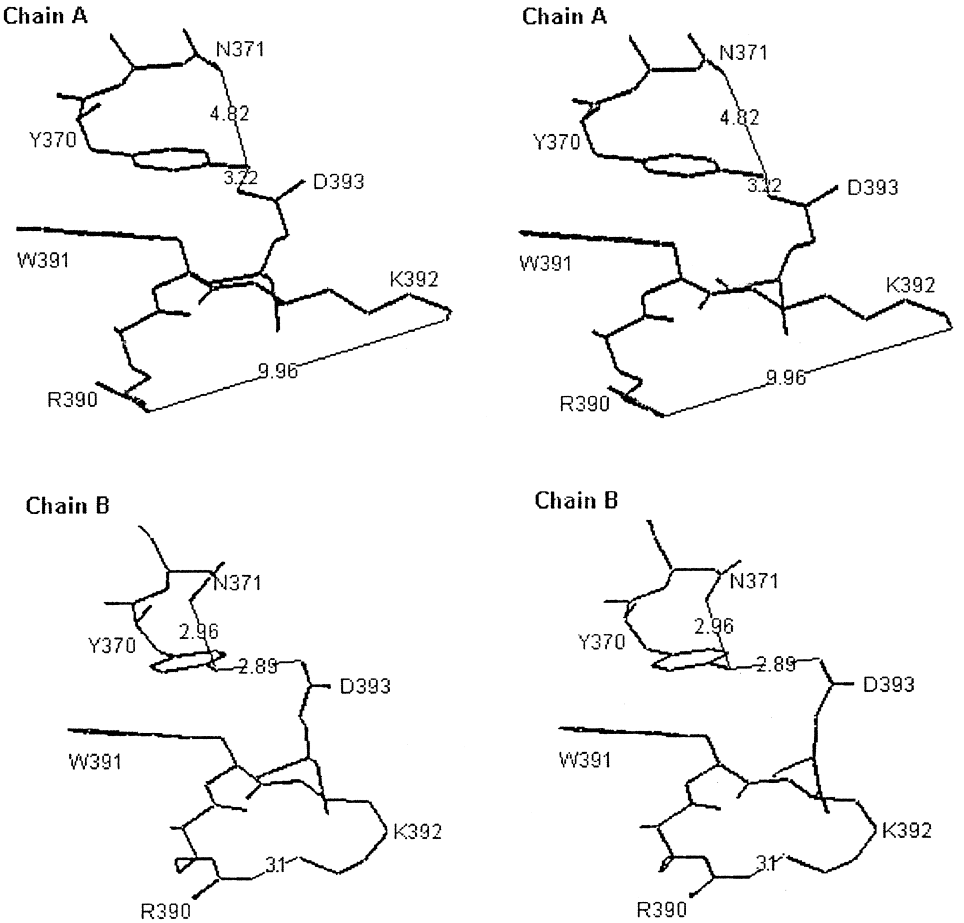

Fig. 3. The Trp^{391}/Tyr^{370} pairs and its environment in the two holoTK subunits (stereo views). The numbers between atoms are the distances (in Å).

The structural homology between TK and the other thiamine-dependent enzymes [11] and the similarity of their properties mentioned above seem to indicate that the conclusions made here for TK are also true for other thiamine-dependent enzymes.

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