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Computer Modeling of Transketolase-Like Protein, TKTL1, a Marker of Certain Tumor Tissues

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Abstract—A computer model of the spatial structure of transketolase-like protein (TKTL1), a marker of certain tumor tissues, has been constructed using the known spatial structure of transketolase found in normal human tissues. The structure of the two proteins at all levels of their organization has also been compared. On the basis of the revealed differences in structures of these proteins, we assume it is unlikely that TKTL1 can be a thiamine diphosphate-dependent protein capable of catalyzing the transketolase reaction.

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Transketolase (TK, EC 2.2.1.1), a thiamine diphosphate (TDP)-dependent enzyme, catalyzes C–C bond cleavage in ketoses (donor substrates) and subsequent transfer of the resulting two-carbon fragments (glycoaldehyde residues) to aldoses (acceptor substrates). The enzyme has been purified from diverse sources. The best-studied enzyme is TK from *Saccharomyces cerevisiae* (sTK) [1].

Until recently, interest in TK from human tissues (hTK) was limited to its medical implications (related to studies of neurodegenerative diseases) [2, 3]. A heterologous expression system of the hTK gene in *E. coli* cells was proposed in 1993 [2]; the spatial structure of hTK obtained by computer modeling in 2008 [4], was resolved in 2010 [5]. TK from normal human tissues is a homodimer with two active centers. Magnesium ions, found in the intact holoenzyme, serve as cofactors of the enzyme, in addition to TDP [6].

Among other factors, interest in human species of TK is accounted for by its suggested role in tumor tissue development. Phosphopentoses present in nucleic acids are largely formed via the pentose phosphate pathway of carbohydrate transformation. In certain tumors, up to 85% of the ribose extractable from nucleic acids originates in the pentose phosphate pathway [7], the key enzyme of which is TK. The growth of tumor tissue is associated with an increase in its TK activity [8]. TK inhibitors cause a dramatic decrease in tumor growth rate [9]. It was therefore evident that TK functioning is directly linked to tumor formation and development.

Relatively recently, transketolase-like protein 1 (TKTL1) was found in tumor, and it is believed to be a form of TK specific for tumor tissue [10-12]. However, it was found that TKTL1 differs from hTK in both primary structure and amino acid composition [5]. The two proteins are 77% homologous at the amino acid level.

Data generated by X-ray crystallography of TDP-dependent enzymes that are now available suggest minor differences between the molecules in either general structure or organization of the active centers [13]. The N-terminal region, in which certain amino acid residues form the active center or are involved in substrate/TDP binding, is among the most conserved portions of the molecule. Note that the 38 amino acid residues (a.a.) whose absence in TKTL1 distinguishes this protein from hTK are also mapped to the N-terminal region.

The presence of TKTL1 in tumor tissue has been used as an early diagnostic marker in progressing tumors since 2005 [10-12, 14]. There are already examples of application of inhibitors of protein synthesis of TKTL1

Abbreviations: hTK, transketolase of normal human tissue; R5P, ribose 5-phosphate; sTK, transketolase of *Saccharomyces cerevisiae*; TDP, thiamine diphosphate; TK, transketolase; TKTL1, transketolase-like protein 1; X5P, xylulose 5-phosphate.

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that can be used as anticancer drugs [12]. It is striking that attempts to find utility for this new form of TK take place in the absence of any data generated by basic research.

In this work, we sought to compare the amino acid sequences of hTK and TKTL1, construct a hypothetic model of the spatial structure of TKTL1, and match the corresponding structural elements (both secondary and tertiary) of the two proteins. The structure and mechanism of action of sTK, which was used as a model enzyme, were studied in detail [15].

RESULTS AND DISCUSSION

Alignment of amino acid sequences of TKTL1 with those of hTK and sTK (Fig. 1; see color insert) demonstrated that 24.56% of the residues are identical. The overwhelming majority of residues forming the active center of sTK were conserved among all three sequences. TKTL1 differed from its counterparts by the absence of a 38-membered peptide containing conserved amino acid residues of the active center. In particular, the TKTL1 molecule lacked two histidine residues involved in binding of the hydroxyl group of the substrate (His69 and His103; the numbering follows that of the sTK sequence [16]), which both fall within the deleted 38-membered peptide.

Note the following substitutions found in TKTL1 and hTK for the conserved residues of sTK: Glu (TKTL1 and hTK) for Cys159; Leu (hTK) and Phe (TKTL1) for Tyr184; Gln (hTK) and His (TKTL1) for Ile191; and Arg (TKTL1 and hTK) for Tyr448.

We used the MODELLER software [17] to obtain hypothetical models of spatial structure of the TKTL1 monomer based on its amino acid sequence and PDB data on the structure of hTK dimer; WinCooT software [18] was further used to build a model for the TKTL1 dimer. MODELLER generated as output files five relatively similar structures. We selected for further work the structure with the minimum value of the molpdf function computed by MODELLER. Thereafter, we optimized the 3D model of the protein. Optimization is required because conformations of loops and side chains are selected randomly in the course of modeling and may not correspond to structures favorable in energy terms [19]. Optimization was performed with account for protein–solvent (water) interactions. This was achieved using AMBER force fields [20]. Thereafter, the two structures (prior to and following energy minimization) were analyzed by the WHAT IF software [21] for comparison with hTK. Specifically, we evaluated the extent to which the structural environment of amino acid residues improved following energy minimization. The model contained certain errors (e.g. amino acid residue overlaps). WHAT IF demonstrated that energy minimization made it possible to reduce the number of overlaps and optimize the structural environment and packing of the residues (data not shown).

Figure 2 shows a computer model of the TKTL1 subunit and known subunit structure of hTK [5].

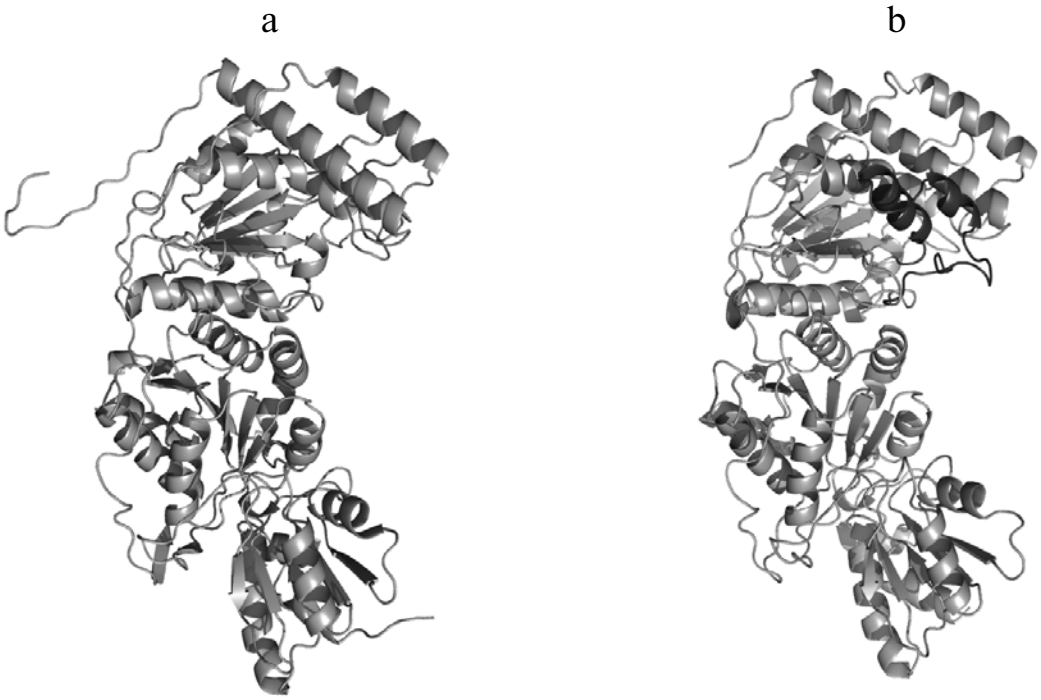

Fig. 2. Computer model of TKTL1 monomer (a) and known structure of hTK monomer (b) [5]. Dark gray indicates deletion of 38 a.a. of the hTK structure.

Comparison of secondary structure elements of hTK and TKTL1, made on the basis of alignment of the amino acid sequences of these proteins (Fig. 1) and the known secondary structure hTK, shows that TKTL1 is a looser molecule than hTK, and it contains cavities.

We further compared structural features of TKTL1 (derived from our hypothetical model) and hTK (reported recently, based on X-ray crystal structure analysis [5]):

– both proteins are homodimers;

– the monomer of hTK consists of three domains and incorporates 52 α-helices, 58 β-strands, and 123 β-turns, and the total number of hydrogen bonds in hTK is 836;

– the N-terminal PP-domain of hTK is involved in TDP binding. In hTK, it comprises amino acid residues 1-276, which form five central parallel β-sheets surrounded by α-helices;

– in hTK the highly conserved loop Gly123-Ser124-Leu125 participates in binding of the aminopyrimidine ring of TDP;

– TKLT1 is devoid of two critical histidine residues that are conserved in other TKs (the substrate-binding His77, involved in the catalytic process, and His110, also required for catalysis);

– TKLT1 contains a mutation in the sequence Gly123-Ser124-Leu125 (Ser124 of hTK is substituted for Trp).

Regarding the structure of active centers, in the hTK molecule the Me^{2+}-binding site contains, in addition to the conserved sequence characteristic of thiamine enzymes (Gly-Asp-Gly, the so-called TDP-binding motif), several residues not found in other TKs (Ser40, Lys75, and Lys244). In TKTL1, the TDP-binding motif itself is mutated, having the structure Ser-Asp-Gly instead of Gly-Asp-Gly (residues 154-156 in hTK).

In hTK, the active center residue interacting with the thiazole ring of TDP is Gln189 (instead of Ile191 in sTK), and Leu125 is located opposite the ring. His258, which is very close to the ring, is probably involved in the catalytic process. In TKTL1, His160 substitutes for Gln189 of hTK.

As shown in Fig. 1, secondary structure elements of hTK and TKTL1 largely coincide. The absence of a large fragment of the amino acid sequence (deletion of 38 a.a.) in the N-terminal PP-domain is a distinguishing feature of TKTL1. This deletion corresponds to residues 76-113 of hTK that form the structure loop–helix–turn–helix–loop and are necessary for the formation of the active center of hTK. Comparison of secondary and tertiary structures of hTK and TKTL1 shows that the C-terminal domain of TKTL1 contains additional β-strands.

The model of TKTL1 constructed in this study and analysis of its primary, secondary, tertiary, and quaternary structures (in comparison with those of sTK and hTK) revealed both similarities and significant differences between the three TK species. The data, together with the fact that TKTL1 lacks an extended peptide fragment in the coenzyme-binding region (which is present, with minor modifications, in all TDP-dependent enzymes characterized thus far), suggest that TKTL1 is incapable of binding TDP and, therefore, is inactive as a catalyst of the TK reaction. The possibility that TKTL1 binds TDP via an alternative mechanism cannot be ruled out. Examples of that kind are known, including some among TDP-dependent enzymes. It is well established that the conserved glutamate, present in active centers of TDP-dependent enzymes, is mandatory for their operation (thiamine catalysis is switched on by the interaction of this residue with the N1′ atom of TDP [22]). However, there is no such residue in the active center of glyoxylate carboxylase (it is substituted by valine), which is still performing its catalytic function [23, 24].

In the literature we have found only one article, the authors of which believe that they have native and recombinant TKTL1, which in their opinion possessed transketolase activity [10]. However, this conclusion cannot be considered convincing, particularly because the article provides no general characteristic of the obtained proteins, there is no protocol for their preparation, no information about homogeneity and specific activity (as long as, in the understanding of the authors, the proteins are catalytically active), there are no actual measurements of experimental curves of transketolase activity (the figure shows the activity expressed in percentage of something unknown), measurement time of enzymatic activity is 24 h (?), with glyceraldehyde-3-phosphate dehydrogenase as an ancillary enzyme no arsenate was added to the system (?), etc. Since the date of publication of that article (since 2005) no other paper has been published in which direct experimental data confirming the conclusions drawn in the above-mentioned article about assessment of transketolase activity of TKTL1 has appeared. So now it is necessary to consider this question open.

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