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Homology modeling of human Transketolase: Description of critical sites useful for drug design and study of the cofactor binding mode

Cristian Obiol-Pardo, Jaime Rubio-Martinez *

Dept. de Química Física, Universitat de Barcelona (UB) and The Institut de Recerca en Química Teòrica i Computacional (IQTCUB), Martí i Franqués 1, E-08028 Barcelona, Spain

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ABSTRACT

Transketolase, the most critical enzyme of the non-oxidative branch of the pentose phosphate pathway, has been reported as a new target protein for cancer research. However, since the crystal structure of human Transketolase is unknown, no structure-based methods can be used to identify new inhibitors. We performed homology modeling of human Transketolase using the crystal structure of yeast as a template, and then refined the model through molecular dynamics simulations. Based on the resulting structure we propose five critical sites containing arginines (Arg 101, Arg 318, Arg 395, Arg 401 and Arg 474) that contribute to dimer stability or catalytic activity. In addition, an interaction analysis of its cofactor (thiamine pyrophosphate) and a binding site description were carried out, suggesting the substrate channel already identified in yeast Transketolase. A binding free energy calculation of its cofactor was performed to establish the main driving forces of binding. In summary, we describe a reliable model of human Transketolase that can be used in structure-based drug design and in the search for new Transketolase inhibitors that disrupt dimer stability and cover the critical sites found.

1. Introduction

Transketolase is a cytosolic enzyme that catalyses the reversible transfer of two carbons, in a glycolaldehyde form, from a ketose donor substrate to an aldose acceptor substrate. It is also the most critical enzyme of the non-oxidative branch of the pentose phosphate pathway. This pathway provides ribose molecules that are an essential metabolite in nucleic acid production. In addition, the ribose necessary for the abnormal proliferation of tumor cells is provided via the non-oxidative branch of the pentose phosphate pathway [1]. In this regard, a metabolic control analysis gave a Transketolase tumor growth control coefficient of 0.9 [1]. Therefore, the inhibition of Transketolase could lead to the development of new anti-cancer drugs, which would decrease the rate of cell division and act at the most critical enzymatic step. Transketolase has also been proposed as a marker for Alzheimer's disease, due to its decreased activity in brain and other tissues of post-mortem patients [2].

Two studies [3,4] have shown that the mechanism of action of Transketolase is mediated by its cofactor thiamine pyrophosphate (TPP), which is coordinated to a divalent metal ion. However, drugs targeting the active site of Transketolase, and which act as cofactor analogs, have poor activity and low selectivity over other thiamine-dependent enzymes such as pyruvate dehydrogenase. oxythiamine [5] or thiamine thiazolone diphosphate [6] are typical examples of this kind of inhibitor, and thus they do not have any pharmacological application.

A lot of work has been devoted to yeast (*Saccharomyces cerevisiae*), *Escherichia coli* and maize Transketolases and their structures have been solved by X-ray diffraction [7–11], revealing important aspects of the functional flexibility, metabolic profile and substrate binding of these variants. Interestingly, three distinct domains were identified in yeast Transketolase: the N-terminal, middle and C-terminal domains [8,9]. The N-terminal domain (residues 1–322) contains the highly conserved TPP binding motif $GDG(X.X)_{25-30}N$, the middle domain (residues 325–538) contains a well-conserved sequence, specifically between residues 475 and 504, while the C-terminal domain (residues 539–680) has been implicated in the formation of a βαβ-fold, with an extended loop between the first β-strand and the α-helix.

However, little research has focused on the human Transketolase protein. A recent study of the human variant reported the critical importance of Asp 155, which is implicated in thiamine pyrophosphate binding [12]. In a similar vein, Du et al. [13] performed high-throughput screening on human Transketolase and found two inhibitors, with a new mechanism of action, but with an unknown binding site. Other authors have reported that some arginine residues (e.g. Arginine 433) are crucial for Transketolase stability

* Corresponding author. Tel.: +34 93 4039263; fax: +34 93 4021231.
E-mail address: jaime.rubio@ub.edu (J. Rubio-Martinez).

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and activity, although this was only identified for the rat variant [14]. More recently, a new class of thiamine analogs were designed as inhibitors of human Transketolase [15–17]. In this regard, it would be useful to determine the structure of human Transketolase in order to conduct further research into structure-based drug design. The present study therefore proposes the first model of human Transketolase, using the 3D structure of the yeast variant as a template and then performing a homology modeling which is subsequently refined through molecular dynamics simulations. This is a general strategy for obtaining a protein structure when no crystal structure is available, and it has been applied to several protein models with noteworthy results [18–20]. Moreover, the dynamic simulation is used to analyze the whole protein–protein interacting surface, which includes some hot spots containing conserved arginines, and to quantify the energetic nature of the thiamine pyrophosphate binding by applying the Molecular Mechanics Poisson–Boltzmann Surface Area (MMPB(GB)SA) protocol [21]. Experimental results [22] suggest that the fundamental interaction between the cofactor and the yeast protein is electrostatic, because a simple molecule of pyrophosphate can compete with TPP for the binding site, while the thiamine fragment of TPP does not compete. In this context, MMPB(GB)SA can provide interesting information, such as electrostatic and van der Waals energies, solvation energies and entropic contributions to binding.

2. Methods

2.1. Construction of the human Transketolase homodimer

The MODELLER 8v2 program [23] was used to construct the human structure. MODELLER is a general program that implements comparative protein structure modeling by satisfying spatial restraints in terms of probability density functions [24,25]. To this end, the homodimer crystal structure of the *S. cerevisiae* yeast Transketolase (pdb code 1AY0 [7]) was used as a 3D template. Although other variants, such as the *E. coli* Transketolase or the maize Transketolase show similar sequence identity to the human protein, the yeast variant was chosen because more information is available about its structure and properties.

Fig. 1 shows the sequence alignment between human and yeast Transketolases that is needed to perform the homology modeling. This was extracted from a multiple sequence alignment, including seven variants, reported by Sundström et al. [26]. The accuracy of the alignment is the most crucial step in assuring the quality of the homology modeling and a multiple sequence alignment is always the preferred approach.

Although the two proteins have low sequence identity (27%) the MODELLER program was applied to generate thirty satisfactory models for each monomer of human Transketolase. The model with the lowest energy and the lowest restraint violation was selected. As regards the secondary structure, STRIDE software [27] was used to predict secondary elements in both the yeast and the best MODELLER model of the human Transketolase (Fig. 1). The secondary structure of the model is similar to the yeast protein, thus confirming the quality of the homology modeling. The major differences are found in fragments containing gaps in the alignment; thus, the alpha helix between residues 269 and 279 in the yeast variant has no equivalent in human Transketolase. Moreover, the long alpha helix between residues 291 and 322 in yeast is constructed as two short alpha helices in the human variant because the alignment also has a gap in this region. These gaps are inevitable, since the human protein is 57 residues shorter than the yeast protein.

As regards the tertiary structure, Fig. 2 shows the two monomers with the best score for the human protein, superimposed over the crystal structure of the yeast variant. As can be

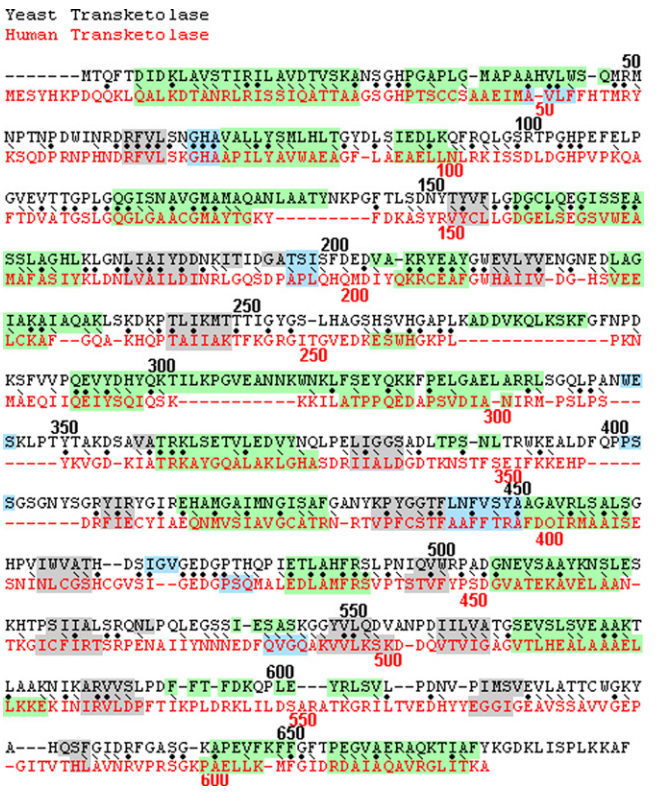

Fig. 1. Sequence alignment between yeast Transketolase (black) and human Transketolase (red) extracted from the multiple sequence alignment of Sundström et al. [26]. Conserved and similar residues are marked with a point and a left slash, respectively. Secondary structure prediction using STRIDE [27] is also shown. Alpha helices are shown in green, 3–10 helices are shown in blue and beta sheets are shown in grey.

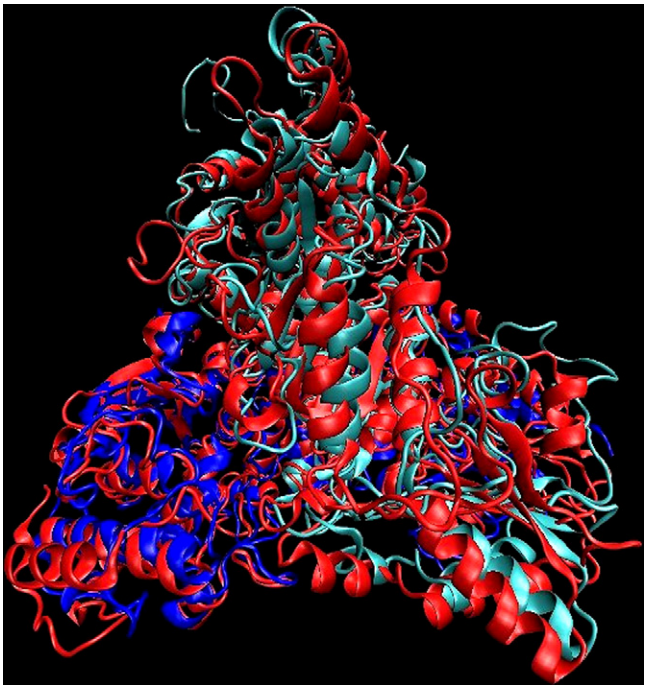

Fig. 2. Superimposition between crystal yeast Transketolase (red) and the two best MODELLER models of human Transketolase (best monomer 1 is shown in cyan, and best monomer 2 is shown in blue). Image created with the VMD software [34].