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Review

Triosephosphate isomerase deficiency: New insights into an enigmatic disease ☆

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ABSTRACT

The triosephosphate isomerase (TPI) functions at a metabolic cross-road ensuring the rapid equilibration of the triosephosphates produced by aldolase in glycolysis, which is interconnected to lipid metabolism, to glycerol-3-phosphate shuttle and to the pentose phosphate pathway. The enzyme is a stable homodimer, which is catalytically active only in its dimeric form. TPI deficiency is an autosomal recessive multisystem genetic disease coupled with hemolytic anemia and neurological disorder frequently leading to death in early childhood. Various genetic mutations of this enzyme have been identified; the mutations result in decrease in the catalytic activity and/or the dissociation of the dimers into inactive monomers. The impairment of TPI activity apparently does not affect the energy metabolism at system level; however, it results in accumulation of dihydroxyacetone phosphate followed by its chemical conversion into the toxic methylglyoxal, leading to the formation of advanced glycation end products. By now, the research on this disease seems to enter a progressive stage by adapting new model systems such as *Drosophila*, yeast strains and TPI-deficient mouse, which have complemented the results obtained by prediction and experiments with recombinant proteins or erythrocytes, and added novel data concerning the complexity of the intracellular behavior of mutant TPIs. This paper reviews the recent studies on the structural and catalytic changes caused by mutation and/or nitrotyrosination of the isomerase leading to the formation of an aggregation-prone protein, a characteristic of conformational disorders.

1. Introduction: major characteristics of triosephosphate isomerase (TPI)

Human TPI is encoded by a single gene located at chromosome 12p13 and is expressed in all tissues. Its amino acid sequence is highly conserved among all known TPI proteins [1,2]. The gene product is a housekeeping enzyme, the physiological function of which is to adjust the rapid equilibrium between the triosephosphates, dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate, produced by aldolase in glycolysis, which is interconnected to lipid metabolism, to glycerol-3-phosphate shuttle and to the pentose phosphate pathway.

TPI is a stable homodimer of two 27-kDa subunits consisting of 248 amino acids. The spatial structure of TPI is one of the most characterized ones as the first example of a $(\beta/\alpha)_8$ barrel fold ("TIM-barrel") [3] (for reviews, see [4,5]). The α-helices and β-sheets are linked by loop regions. Three loops of the N-terminal half of the molecule are involved in the intersubunit interactions, other three ones participate in the active site [1]. One of them is a flexible loop, the movement of which is necessary for providing the so called "closed" (liganded) state of the enzyme [6]. The 3D structure of human recombinant isomerase was also obtained by crystallography at a resolution of 2.8 Å [1]. Three residues, Lys13, His95 and Glu165, form the active site. However, it was proposed that residues indispensable for enzyme activity exist throughout the C-terminal region of the protein, with the possible exception of the ultimate few amino acids.

TPI is catalytically active only in its dimeric form. The crucial role of the dimeric form both in the catalytic function and the stability was clearly demonstrated by Mainfroid et al. [7] by producing recombinant enzymes with "artificial" mutations Met14Gln and/or Arg98Gln. These mutations at the dimer interface decreased the stability of the isomerase due to its dissociation into inactive monomers.

2. TPI deficiency

TPI deficiency is the most severe glycolytic enzymopathy, the only one which is lethal, frequently in early childhood. In spite of the efforts to understand the basis of this disease, our knowledge is rather limited due to its rarity and/or the failure of its recognition. Homozygotes and compound heterozygotes manifest not only congenital hemolytic anemia, as in the case of more frequent disorders of glycolytic enzymes, but their symptoms include neurological dysfunctions as cardiomyopathy and progressive neuromuscular impairment. No effective therapy is available for TPI deficiency.

Abbreviations: TPI, triosephosphate isomerase; DHAP, dihydroxyacetone phosphate; AD, Alzheimer's disease; AGE, advanced glycation end product; Aβ, amyloid β-peptide

☆ This paper is dedicated to Prof. Susan Hollan's memory.

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TPI deficiency is an autosomal recessive multisystem genetic disorder, characterized by decreased enzyme activity, which is accompanied by the elevation of the substrate, DHAP, level. This phenomenon is the most distinct in erythrocytes, where the multiple consumption of DHAP does not hold; thus, in the case of the lack of TPI activity it is a dead-end product.

The most frequent missense mutation detected in TPI-deficient patients occurs at codon 104 in the TPI gene (Glu104Asp mutant), which accounts for approximately 80% of mutant alleles within patients with clinical TPI deficiency [2,8]. Glu104Asp arose as a single mutation in a common ancestor of the affected families [2,8]. This mutation is not only the most common but also causes the most severe symptoms. Some additional mutations have been identified so far with infrequent occurrence, mostly in compound heterozygotes, coupled with Glu104Asp mutation [2,9]. A unique case is that of the compound heterozygote Hungarian brothers, identified by S. Hollan; both brothers carry the mutations of Phe240Leu and Glu145Stop; however, neurological symptoms have developed only at the younger one [10].

Extensive genetic and biochemical work was carried out to reveal the basis of this disease. The studies at atomic and molecular levels focused primarily on the structural background of the decreased enzyme activity. At system level, the main questions arisen are whether the decreased activity can maintain the normal glycolytic flux; why it results in the extreme elevation of the DHAP level; and how the mutation is connected with the neurological symptoms.

The doyen of TPI deficiency research, A. Schneider, reviewed the accumulated data from hematological point of view and discussed the genetic background and molecular aspects of the disease, too [2]. More recently, we have summarized the molecular, structural and energetic aspects of the disease, providing a system level analysis of the deficiency, as well [9].

A couple of papers, using yeast, fly and mouse models of TPI deficiency, have been recently published, which significantly contribute to our understanding the structural and functional consequences of the mutations. A view seems to be emerging that the reduction in the stability of the dimeric enzyme due to the mutations at the subunit interface can be a crucial component in the etiology of the illness. The new results support our former view that TPI deficiency is rather a conformational than a metabolic disease, although accumulation of toxic metabolites might also play a role in its clinical manifestations.

3. Functional and structural consequences of the mutations

The human pathogenic mutations are not restricted to a specific domain or region of the enzyme. Bioinformatic analysis, based on the 3D structure of the *wild-type* enzyme, was used by Schneider [2] to explain the structural and catalytic properties of the mutant enzymes observed in the patients' hemolysates. He mapped the amino acid residues as well as the first- and second-degree contacts of all of the residues comprising the functional domains of the enzyme. Mutation sites coupled with the *substrate binding site* (the active center) would manifest activity decrease, while those in or interacting with the *dimer interface* are expected to exhibit molecular instability manifesting as thermolability coupled with catalytic abnormalities.

Cys41Tyr, Gly72Ala, Ile170Val, Val231Met and Phe240Leu mutations were suggested to interact with the substrate binding site [2] (Table 1). Among these amino acid residues, Ile170 is not only in the neighborhood of Glu165 of the active site but is also part of the flexible loop, the conformation of which alters during the ligand binding [6]. Indeed, significant activity decrease was observed in all these cases [9].

Mutations Cys41Tyr, Ala62Asp, Gly72Ala, Glu104Asp and Val231-Met were supposed to influence the contact surface of the dimeric TPI [2] (Table 1). The involvement of amino acids Glu104 and Gly72Ala in the stabilization of dimeric form is clearly supported by the 3D structural model of the wild-type enzyme (Fig. 1), which explains the significant instability and decreased activity of these mutants. The effects of the Cys41Tyr and Val231Met mutations, which were suggested to influence both the dimer interface and the active site, are not so trivial; however, beside the activity decrease, thermolability was also demonstrated in the four cases when investigated. (No stability study has been carried out in a recently published case with Ala62Asp mutation.)

However, it seems that the static 3D model, based on crystal structures of the wild-type enzyme, does not capture all the functional relationships between the mutations and catalytic defects in the case of TPI deficiency. Thermolability was also found for the Phe240Leu [11] and Gly122Arg [12] mutations, which were not suggested to affect the dimer interface. Moreover, in two cases (occurring only in

Table 1
Characterization of missense TPI mutations of patients and of animal models.

Mutation	Effect of the mutation on[a]		Recombinant enzyme		Yeast[b] model		Hemolysate [c]		Neurological disorder[c]	Reference
	Substrate binding	Dimer interface	Activity loss	Instability	Activity loss	Instability	Activity loss	Instability		
Met Init Lys	Nonsense mutation		n.d.	n.d.	Not viable		+++	n.d.	++	[44]
Cys41Tyr	+	+	n.d.	n.d.	−	−	+	+	+	[8]
Ala62Asp	−	+	n.d.	n.d.	n.d.	n.d.	++	n.d.	n.d.	[45]
Gly72Ala	+	++	n.d.	n.d.	n.d.	n.d.	++[h]	++ [h]	?[h]	[46]
Glu104Asp	−	++	− [f]	++ [f]	−	++	+/++	++	++	[14]
Gly122Arg	−	−	n.d.	n.d.	−	−	−	+	?[h]	[12]
Val154Met	−	−	n.d.	n.d.	n.d.	n.d.	++[h]	++[h]	?[h]	[46]
Ile170Val	++	−	n.d.	n.d.	+	−	++	−	−	[8]
Val231Met	+	+	n.d.	n.d.	n.d	n.d.	+	+	+	[47]
Phe240Leu	+	−	+ [g]	+ [g]	−	−	++	+	+/−	[10]
Asp49Gly [d]	−	++	n.d	n.d.	n.d	n.d.	+[d]	++[d]	n.d.	[25]
Met82Thr [e]	−	++	n.d	n.d.	n.d	n.d.	n.d	n.d.	+[e]	[18–20]

n.d. – no data; −, none; +, moderate; ++, severe; +++, complete loss.
[a] Based on Schneider [2].
[b] From Ralser et al. [16].
[c] From Schneider [2] and Orosz et al. [9].
[d] Mouse model [25].
[e] Fly model [18–20] in which Met80 corresponds to human Met82.
[f] From Rodríguez-Almazán et al. [13].
[g] From Orosz et al. [11].
[h] No patients. Found in a population survey in healthy heterozygotes. Indirect activity and stability data.