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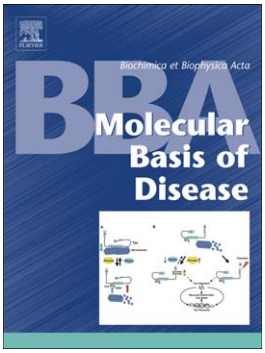

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Triosephosphate isomerase deficiency: new insights into an enigmatic disease[1]

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

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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.

Keywords: neurodegeneration, enzymopathy, conformational disease, glycolysis, methylglyoxal, advanced glycation end products (AGEs), oxidative stress, animal models

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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

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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.

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

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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 Val231Met 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

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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 heterozygotes) no interaction with a functional domain was suggested (cf. Table 1) and the reported deficiency of these mutations remains unexplained. There are two plausible explanations for these discrepancies: (i) some mutations may cause local or global conformational changes that are not evident using the static model; and (ii) protein activity and stability were typically assayed in hemolysates of the patients where intracellular associations could further alter either structure or function of the enzyme.

4. New knowledge from studies with recombinant mutant enzymes

Despite extensive work that has been carried out on the mutant enzymes in hemolysates, until very recently only the Phe240Leu mutant TPI was studied in recombinant form [11]. This kind of investigation can provide direct information whether intrinsic alterations of the enzyme or external factors are responsible for the discrepancies between prediction and measurements made in cell extracts.

Now, the characteristics of the Glu104Asp recombinant enzyme have been ascertained and compared with those of the recombinant wild type human TPI [13]. Direct evidence for the structural changes in the dimer interface of TPI due to the Glu104Asp mutation was obtained from the 3D structure of the recombinant mutant enzyme by x-ray analysis (at 1.85 Å resolution) [13]. Formerly, it was proposed on the basis of the crystal structures of *wild type* TPI [1, 3] that a counterbalance of charges would lower the stability of the dimer, with an eventual perturbation of the local structure of the active site [1, 14]. However, the structure of Glu104Asp *mutant* human TPI [13] revealed that the geometry of the catalytic residues was not modified and that the most important consequence of the Glu104Asp mutation is the disruption of a conserved water network that spans the dimer interface and appears to be essential for maintaining the stability of TPI dimers. This mutation, affecting the dimer interface, does not influence

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the catalytic activity *per se*; the kinetic parameters of the mutant and wild type recombinant enzymes are nearly identical [13]. Yet, decreased TPI activity was detected in the patients' hemolysates (cf. Table 1), probably due to the dissociation of the enzyme into inactive monomers. This idea was further supported by measurements of the stability and reactivation of the wild type and Glu104Asp mutant recombinant enzymes from unfolded monomers, which revealed that the formation of active dimers from unfolded inactive monomers was impeded in the case of the mutant enzyme as compared to that of the wild type [13].

Moreover, thermal denaturation data obtained by circular dichroism spectroscopy, differential scanning calorimetry and enzyme activity measurements revealed the extreme thermolability of the Glu104Asp mutant [13]. Namely, it was fully inactivated in 4 min at 48 ºC, while in the case of the Phe240Leu recombinant mutant it occurred in 30 min at 52 ºC [13, 11]. In addition, the two different mutations resulted in different thermal denaturation routes determined by differential scanning calorimetry; Phe240Leu mutant has two transitions, whereas the Glu104Asp mutant has only one, at a significantly lower temperature. These differences can also be interpreted on the basis of the distinct sites of the mutations. The replacement of Glu at position 104 counteracts with the intersubunit interaction, while, according to our molecular dynamics simulation, a distinct conformational state in the active-site region was seen due to the Phe240Leu mutation; but it did not extend to the subunit contact surface [15]. Although the Phe240Leu mutant is also somewhat thermolabile in comparison with the wild type, the local conformational changes may not be extended to the dimer interface and do not influence significantly the dimerization. However, this mutant is expected to influence the catalytic activity [2, 15]. Indeed, the activity of the recombinant Phe240Leu mutant was 30% of that of the wild type [11].

5. New models for studying TPI deficiency

Research on TPI deficiency recently has entered a new stage by adapting new model systems, such as Drosophila, yeast strains and genetically modified mouse, which supply mutation-related experimental data for the in vivo behavior of TPI mutants. The high degree of TPI sequence conservation from bacteria

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to human and the high degree of structural similarity observed in TPI crystal structures [1] allow one to evaluate pathologically relevant information on the effects of specific mutations in TPI from these models. This is an important issue since patients' symptoms often depend on their genetic background and environmental factors, which are, of course, not present in experiments using recombinant enzymes.

5.1. Yeast models

Ralser et al [16] exploited a well established yeast system, which allowed them to decrease these additional effects, and to study the enzymatic and structural properties of wild type and pathogenic TPI variants and to characterize the effect of the mutations at different regions (catalytic site or dimer interface) of TPI.

For studying the catalytic properties they used a single gene replacement approach. A *Δtpi1* strain deleted for the yeast *TPI1* gene was generated and transformed for the expression of human wild type or six representative pathogenic TPI mutants. With the exception of a TPI with a nonsense mutation (Met Init Lys), all yeast strains containing mutant TPI were viable on glucose media demonstrating the functional activity of these enzymes. The *Δtpi1* cells expressing the mutant TPIs, Cys41Tyr, Glu104Asp, Gly122Arg or Phe240Leu, exhibited catalytic activity *in vivo* that was comparable to that of the cells expressing wild type protein. This observation differs from that obtained with hemolysates of patients and with recombinant Phe240Leu mutant but is in agreement with that obtained with the recombinant Glu104Asp TPI. The Ile170Val mutant showed a strong reduction in catalytic activity (Table 1), which was not due to reduction of enzyme level but the decreased enzymatic activity *per se*. This observation resembles the one obtained by prediction based upon 3D structure and by measurement of activity in hemolysate (Table 1). The residual activity (about 30%) was still sufficient to suppress the growth defect of *Δtpi1* yeast cells on glucose medium [16].

To investigate whether the mutations within the TPI gene affect the dimerization behavior of the protein, the yeast two-hybrid system was used. The yeast strain *L40ccua* was transformed with a bait construct encoding wild type TPI in combination with prey constructs encoding the wild type or one of the

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above mentioned mutant TPIs. The relative activity of the *lacZ* reporter gene, which indicates the relative strength of a protein-protein (in this case the subunit-subunit) interaction [17], was measured by using a β-galactosidase activity assay. It significantly decreased (less than 20% of the control) only in yeast co-expressing the wild type (bait) and Glu104Asp (prey) TPI demonstrating the impaired dimerization. Similar effect was established when the formations of the homodimeric Glu104Asp or its heterodimers with the other mutants were tested. The only exception was the Ile170Val mutant which stabilized somewhat the heterodimer [16]. These results may also explain the fact that TPIs of the compound heterozygote patients carrying Glu104Asp and another mutation are also thermolabile except the combination of Glu104Asp and Ile170Val (cf. [9]).

The results indicate that the yeast system, although did not mimic all consequences of the mutations, was suitable to model the consequences of the two most characteristic ones: the aberrant dimerization and the decreased activity of the Glu104Asp and the Ile170Val mutants, respectively.

5.2. Drosophila model

The architecture of the fly nervous system is similar to that of mammals, thus fly models are often used to study the progression of various diseases. By using forward genetic screens, two independent research groups identified the same TPI deficient fly mutant (named *sugarkill* or *wasted away*) with phenotypes showing analogous symptoms and characteristics to those of human TPI deficiency, including progressive locomotor impairment, temperature sensitivity, vacuolar neuropathology, and severely reduced life-span [18, 19].

This recessive hypomorphic TPI mutant carries the Met80Thr mutation; Met80 corresponds to Met82 in human TPI [18, 19]. This mutation affects a conserved methionine residue that resides at the TPI dimer interface (Table 1). Therefore, not surprisingly, the above mentioned characteristic features of the mutant fly show the best parallel with the Glu104Asp human mutation. Mutation at the dimer interface results in the instability of the isomerase leading to the proposal that dimer instability underlies the temperature sensitivity observed in the flies [18]. Despite this logic suggestion, further investigation by

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the same group showed that the mutant protein maintained its dimeric state when the temperature of the whole fly extract was elevated [20]. This unexpected stability of the mutant isomerase could be due to its heteroassociations to unrelated proteins as demonstrated earlier [21]. Biochemical studies on mutant Drosophila models demonstrated that the phenotype was not the result of impaired bioenergetics [18, 19], which resembles the human data [2, 10, 22]. Although markedly reduced lactic acid level was detected [18], ATP [19] and phosphocreatine [18] levels remained normal and increased, respectively.

The mRNA and protein levels of TPI were investigated in TPI^{sugarkill} mutant fly [20] at room temperature and at 29 ^{o}C (a physiologically relevant temperature for Drosophila). The level of mRNA was not reduced at any temperature examined, however, the protein level was reduced in temperature-dependent manner: 73% and 98% reduction was detected at room temperature and at 29 ^{o}C, respectively, as compared to that of the wild type fly. The severity of this reduction was antagonized by decreasing temperature, consistent with other phenotypes observed in TPI^{sugarkill} mutant. This phenomenon could be attributed to the instability and/or the active proteolytic degradation of TPI^{sugarkill} protein. Indeed, genetic inhibition of the 20S proteasome core resulted in modest increase in TPI^{sugarkill} protein level [20], which may indicate the misfolding of TPI by this mutation.

5.3. Mouse model

A breakthrough could be expected in TPI deficiency research if a stable mouse model was established. The high degree of sequential identity (95%) and structural similarity of the mammalian enzymes, the expected resemblance of the pathological consequences of the mutation in mouse and human; the availability of blood, and especially, of brain samples, are obvious advantages in comparison with yeast or fly models.

Former attempts generally resulted in homozygous lethal mutations due to a total lack of TPI activity [23] or mice which did not show any symptoms but decreased enzyme activity [24]. Very recently, a TPI mutant, *Tpi1*$^{\text{a-m6Neu}}$, with approximately 13% residual enzyme activity in blood of homozygotes compared with that of the wild type has been produced by triethylenemelamine treatment

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[25]. The homozygous animals are viable with full fertility and complete penetrance of the mutation. Sequence analysis revealed an Asp to Gly substitution at codon 49. This residue is 100% conserved in mammals. Asp49 was suggested to directly participate in the dimer interface [2]. As expected, the mutant TPI exhibited molecular instability manifested as thermolability. TPI heat lability of erythrocytes at 50 ºC in homozygous mutants is so strong that the activity is reduced to zero after only few minutes of incubation. This behavior fully resembles that of the human Glu104Asp mutant.

The deficient mice show hemolytic anemia in homozygous condition. Accordingly, significant deviations from the wild type were observed for the values of hematocrit, hemoglobin, the number and mean corpuscular volume of red blood cells, mean corpuscular hemoglobin concentration and spleen weight [25].

The activity decrease was much less in other tissues, especially in liver, kidney or brain (about 50%), than in blood [25]. This phenomenon resembles the case when significantly higher TPI activity was measured in the lymphocyte lysates of compound heterozygote patients carrying the Phe240Leu and the Glu145Stop mutations than in their hemolysates [10, 26]. A possible explanation for this difference is that the decreased enzyme activity/level caused by the instability of the mutant TPI is not restored due to the lack of synthesis in mature erythrocytes, whereas enzyme synthesis would maintain near-normal enzyme activity in other tissues/cells. Therefore, the data obtained with the mouse model carrying Asp49Gly mutation in TPI reinforced the notions based on data obtained in other intracellular milieu (with recombinant enzymes, human blood sample and yeast model) that the mutation at the dimer interface results in instability of the dimeric enzyme causing its dissociation into inactive monomers with concomitant impairment of the enzyme activity.

4. Nature of mutations *vs.* clinical symptoms. TPI deficiency as a conformational disease

The data accumulated in the past seems to show a correlation between the nature of the mutations and the clinical symptoms. A good example for this issue is the propositus carrying the Ile170Val mutation in the *active site region*, who is free of neurological symptoms. Therefore, it was suggested that

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structural changes rather than abnormal catalysis might play an important role in the generalized clinical manifestations of TPI deficiency [8]. The new data corroborate this hypothesis, especially concerning the significance of the altered quaternary structure, i.e. the mutation induced instability of the TPI dimer. Comparing the clinical symptoms with the data of cell extracts, of the yeast model system, of recombinant enzymes and of structural modeling, we can conclude that the aberrant dimerization is the most important component in the molecular basis of the illness. The rather the mutation influences the dimer interface the more serious the symptoms are.

Patients carrying mutations which do not influence the dimerization process (Gly122Arg, Ile170Val, Phe240Leu) suffer from a moderate form of TPI deficiency, and are generally free of neurological symptoms (Table 1). In the case of the Phe240Leu mutant, only one of the two compound heterozygote patients developed neurological symptoms, but even he is in stable condition in adulthood [10]. (Their other mutation is a nonsense one at codon 145 (Glu145Stop) leading to a truncated TPI.) Although Gly122Arg and Phe240Leu TPIs are somewhat thermolabile, it may be caused by some alterations in the secondary/tertiary but not in the quaternary structure.

Cys41Tyr and Val231Met mutations probably influence the dimerization process, as suggested by prediction based on the 3D structure of the native protein; the enzymes are thermolabile, and the patients suffer from neurological disorder. However, they have prolonged life-span (> 8 years) in comparison with the Glu104Asp homozygote patients dying in infancy or early childhood. In this latter case, it was unambiguously shown by various methods (prediction, X-ray structure of the mutant TPI, *in vitro* stability assays, yeast two-hybrid system) that the aberrant assembly of the mutant TPI subunits is coupled with its inactivation and finally, through not yet revealed steps, with the death of the patients. As we suggested previously, it might be a consequence of the formation of toxic aggregates from the misfolded protein in the brain. ("TPI deficiency as conformational disease" hypothesis [9].) Taking into consideration that apart from being necessary for enzyme activity, dimerization ensures also the conformational stability of TPI (the folded monomer is only weakly stabilized as compared to the unfolded monomer) [7], the unstable monomer might display high aggregation ability. Our suggestion has been further strengthened

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by recent data published by Guix et al. [27] (see later). (Further human mutations, which are predicted to influence the dimer interface, has been found only in a population survey in a healthy heterozygote (Gly72Ala), or in a case not yet studied in details (Ala62Asp).)

Decreased, even significantly, catalytic activity *per se* does not seem to lead to fatal consequences if the enzyme has stable quaternary structure. It is not surprising if we take into account the very high catalytic activity of the native enzyme. As we and others showed, even the lowest TPI activity detected in the hemolysate of the patients has to be enough to ensure normal glycolytic flux [22, 26, 28]. In accordance with this, experimentally detected alterations in glucose utilization, ATP and lactate production were found to be unimpressive [2, 10, 22]. However, two observations should be noted. First, that the equilibrium of triosephosphates is not achieved in mutant erythrocytes resulting in a significantly elevated DHAP concentration in all cases [26]. Second, the relative activities of the mutant enzymes (compared to that of the wild type one) in hemolysates (2-30%) were always less than those detected with recombinant enzymes or in the yeast model system (30-100%). This phenomenon was attributed to the decreased stability of the mutant enzymes [29] and/or to intracellular interactions altering their structure and function [11, 30].

As we pointed out, a major hurdle to elucidate the pathomechanism of TPI deficiency is the lack of brain tissues available for experimental purposes [9]. The presence of the mutant protein might result in the formation of toxic protein aggregates and/or the impairment of energy metabolism. The first possibility has arisen because no energy deficit has been demonstrated either in patients or in the Drosophila model. However, neither of these possibilities has been tested in brain. It is well demonstrated that in other neurodegenerative diseases, such as Alzheimer's (AD), Parkinson's or Huntington's diseases, unfolded or misfolded proteins form aberrant protein–protein interactions that lead to the formation of toxic protein aggregates causing neuronal dysfunction. The accumulation of unfolded or misfolded proteins could impair energy metabolism by mechanisms that are not fully understood. The simultaneous investigation of these two aspects has been carried out recently using mouse models for Huntington's disease: the affected brain regions with pathological inclusions were identified by immunohistochemistry

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and used for biochemical analysis of the ATP-producing systems in the cytosolic and the mitochondrial compartments [31]. Similar neurological and energetic analysis of TPI mutant mice can be promising for the clarification of the pathomechanism of TPI deficiency.

7. Relationship of TPI deficiency with other conformational diseases

TPI was identified as one of the main proteins nitrotyrosinated by nitric oxide synthase in AD patients [27, 32-34] and in AD transgenic mice [27]. Higher levels of nitro-TPI were detected in immunoprecipitates from hippocampus and frontal cortex of AD patients, compared with healthy subjects [27]. Nitro-oxidative damage, induced by amyloid β-peptide (Aβ), promoted the nitrotyrosination of TPI in human neuroblastoma cells. Due to the nitration of tyrosines 164 and 208, close to the catalytic center and the flexible loop, TPI nitrotyrosination decreased about ten times the enzyme activity and triggered the production of the highly neurotoxic methylglyoxal, as a by-product, *in vitro* [27]. Human embryonic kidney cells overexpressing double mutant TPI modified at these two tyrosines (Tyr164Phe and Tyr208Phe), which mimicked the effect of nitrotyrosination on loop stability, showed high methylglyoxal production. In accordance with these findings, methylglyoxal levels were significantly higher in AD brains than in healthy controls [27]. Glyoxalases cannot efficiently detoxify the accumulated methylglyoxal, which leads to enhanced production of toxic *advanced glycation end products* (AGEs). The accumulation of glycation adducts results in deleterious consequences including *oxidative stress* (accumulation of reactive oxygen species) (Figure 2), DNA damage, and apoptosis, in various diseases [35], especially in the pathogenesis of degenerative neurological disorders [36]. TPI was found among proteins become oxidatively modified in early [37] and late [38] AD as well. These findings correlate with the widespread glycation in the cortex and hippocampus from AD transgenic mice [27].

These aspects have been arisen in TPI deficiency as well, where the increased DHAP level leads to an accumulation of methylglyoxal, *composed from the accumulated DHAP* non-enzymatically, and results in the consequent enhanced level of AGEs [39] (Figure 2). Additionally, DHAP itself is also a glycating agent (Figure 2), and it has been shown recently that it is capable of condensing not only with proteins

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altering their structure or function but also with GTP [40]. The above mentioned results by Guix et al. [27] suggest that extensive methylglyoxal production can also occur directly due to the functional defect of TPI. Moreover, TPI nitrotyrosination probably occurs also in TPI deficiency since an 8-fold increase in the mRNA level of nitric oxide synthase [26] (Figure 2), the enzyme responsible for nitration, and a 15-fold increase in urinary 3-nitrotyrosine [39] were found in a Hungarian TPI deficient patient carrying Phe240Leu and Glu145Stop mutations, the one with neurological symptoms. Increased chronic oxidative stress was also detected in the same patient [41].

It has also been demonstrated that nitrotyrosination induces the aggregation of TPI [27] (Figure 2). Furthermore, the nitrotyrosinated TPI forms large structures with β-sheet folds that are induced in neurons cultured *in vitro*, in the presence of Aβ, and, importantly, are observed in cortex from AD brain. The large size of nitrotyrosinated TPI aggregates make them resistant to degradation by the proteasome, and these aggregates bind tau monomers and induced tau aggregation to form paired helical filaments, the characteristic intracellular hallmark of AD brains [27]. TPI co-localizes with tau in AD, but not in healthy cortex and immunoprecipitates tau in both AD brains and transgenic AD mice.

These findings corroborate the earlier ones that two fragments of TPI are structurally homologous to the Aβ [42]. It was also shown that one of these segments, with partial sequence homology to the Aβ, is able to form amyloid *in vitro*. Moreover, TPI was recognized in pig brain, mainly in the vessel walls of the hippocampus, by antibodies raised against Aβ [43]. It was suggested that the crossreactivity between Aβ and TPI was a synergic consequence of a partially homologous amino acid sequence and their similar 3D conformational epitopes.

One can hypothesize that modifications/mutations influencing the structure of TPI may strengthen its likely inherent tendency for aggregation. It was shown that some mutations in TPI, including Glu104Asp and in less extent Phe240Leu, make it more prone to associate with brain microtubules, which influences their polymerization and decreases the activity of the enzyme [11, 30].

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Altogether, the results reviewed in this paper show that the development of the disease named as TPI deficiency from some respects resembles conformational diseases which are initiated by misfolded proteins. Investigations using TPI deficient mice and *post mortem* human brain samples can further support this view and will serve as an effective framework in order to understand the nature of TPI deficiency at molecular level.

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Abbreviations

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

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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]

[a] – Based on [2]; [b] – from [16]; [c] – from [2, 9]; [d] – mouse model [25]; [e] – fly model [18-20] in which Met80 corresponds to human Met82; [f] - from [13]; [g] - from [11]; [h] - No patients. Found in a population survey in healthy heterozygotes. Indirect activity and stability data. n.d. – no data;
-, none; +, moderate; ++, severe; +++, complete loss.

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Legends to the Figures

Figure 1. Schematic ribbon diagram of the crystal structure of recombinant wild type human TPI based on [1]. Lys13, His95 and Glu165 are the active site residues (black). Amino acid residues substituted by inherited mutations in human patients and heterozygous carriers are Cys41 (1A, orange), Ala62 (1B, yellow), Gly72 (1B, blue), Glu104 (1B, red), Gly122 (1B, lilac), Val 154 (1A, yellow), Ile170 (1A, blue), Val231 (1A, red) and Phe240 (1A, lilac). Asp49 (1B, orange) and Met82 (1B, green) (in fly Met80) were mutated in mouse and fly models of TPI deficiency, respectively. The figure was generated by the DeepView/Swiss-PdbViewer v3.7 software (http://www.expasy.org/spdbv/) using the recombinant human TPI structure (1HTI pdb file).

Figure 2. Tentative scheme of development of neurodegeneration in TPI deficiency. wTPI, wild type TPI; mTPI, mutant TPI; GAP, glyceraldehyde-3-phosphate; ROS, reactive oxygen species; NO, nitric oxide.

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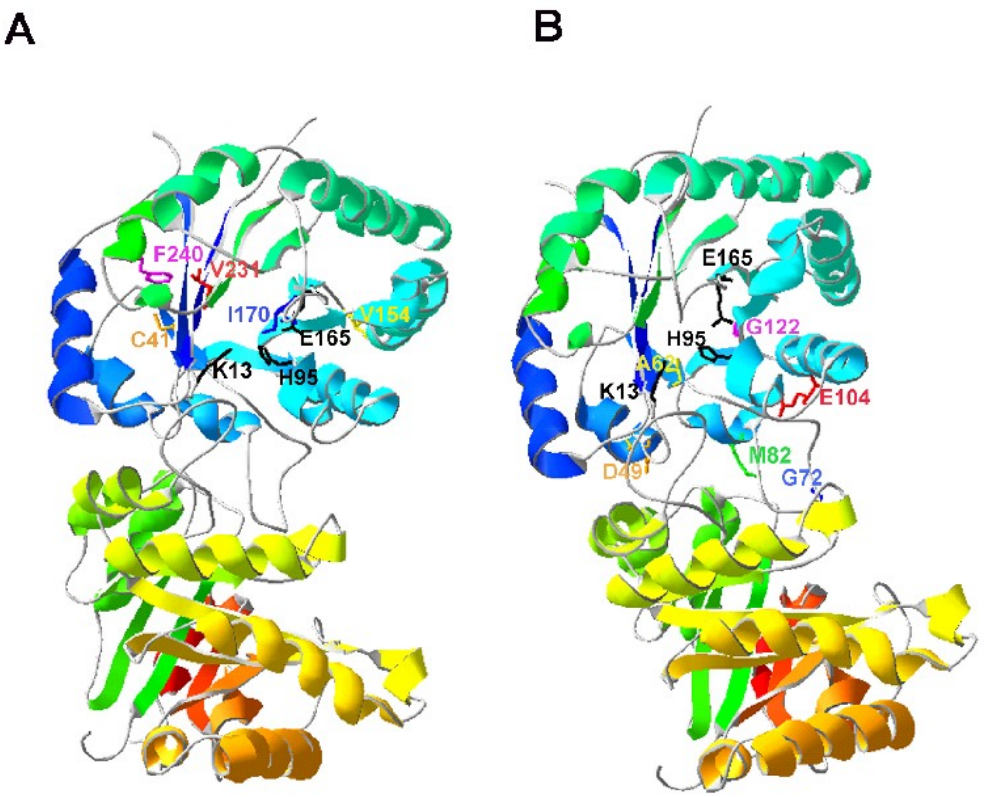

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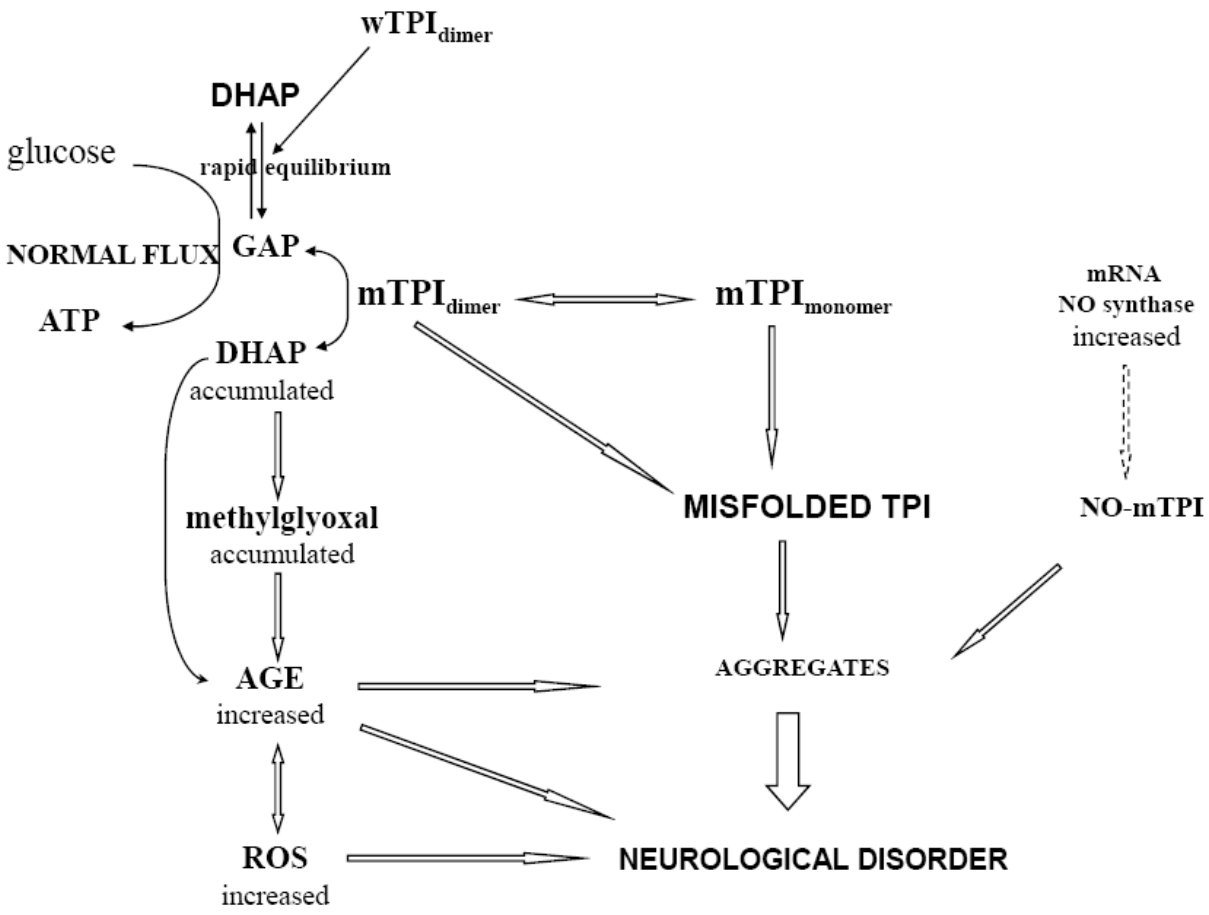
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