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Phosphoglucose isomerase – "portrait of the protein with many faces"

Izomeraza fosfoglukozowa – „portret białka o wielu twarzach"

Phosphoglucose isomerase (D-glucose-6-phosphate aldose-ketose-isomerase; glucose-6-phosphate isomerase; phosphohexose isomerase; PGI; EC 5.3.1.9) catalyzes the reversible isomerization of glucose-6-phosphate (G6P) to D-fructose-6-phosphate ester (F6P).

BIOLOGICAL FUNCTIONS OF PGI

PGI plays a crucial role in the phosphorylated sugars metabolism: glycolysis, phosphate pentose pathway and gluconeogenesis. Hence, phosphoglucose isomerase has been sometimes described as a "workhouse" enzyme of sugar metabolism [10]. PGI is involved in biosynthesis of arabinogalactan (component of mycobacterial cell wall) [1, 25]. This enzyme performs the key role in the governing carbon availability for the synthesis extracellular polysaccharides (EPS) of bacterial biofilms *in vivo* [37].

PGI has a number of other "moonlighting" functions [19, 20]. Jeffery explained that "moonlighting" refers to a single protein that has multiple independent functions, which are not the effects of gene fusion, splice variants or the presence of multiple proteolytic fragments [20]. The function of these type proteins may be a consequence of changes in cellular localization, cell type, oligomeric state, concentration of a ligand, substrate, cofactor or product. In many cases, the protein uses a combination of these methods to switch between functions [19].

PGI with novel lysyl aminopeptidase activity (PGI-LysAP) was characterized from the human pathogen *Vibrio* (*V.) vulnificus* [28, 29] and was also detected in *V. cholerae* and *V. parahemolyticus* [28].

PGI revealed specific inhibitory activity towards a myofibril-bound serine proteinase (MBSP) from crucian carp (*Carassius auratus*) [33] and white croacker (*Argyrosomus argentatus*) [8].

In mammals, it was shown that several proteins such as neuroleukin (NLK), autocrine motility factor (AMF), and differentiation and maturation factor (DMF) are closely related, supposedly even identical to PGI [34]. GPI as NLK is a neutrophic factor for sensory and specific embryogenic spinal neurons [9, 12, 13]. NLK is secreted by lectin-activated T lymphocytes and it promotes secretion of immunoglobulins by cultured human peripheral blood mononuclear cells [12, 13, 39]. PGI was also identified as the SA-36 antigen involved in sperm agglutination. It was found that SA-36 cDNA displayed > 99% homology

to human GPI/NLK [39]. In fact, only three SA-36 amino acids (aa) at positions 158, 426 and 436 were not identical to 558 aa of human GPI/NLK [39]. AMF is secreted by tumor cells to promote cell motility and proliferation [42]. An additional biological function of GPI as a DMF is the ability to mediate the differentiation of human myeloid leucemic HL-60 cells to terminal monocytic cells [38]. A positive correlation between anti-GPI autoantibody and the arthritis disease in humans have been suggested [31]. Inherited deficiency of human GPI affects mostly erythrocytes causing hereditary nonspherocytic hemolytic anemia (HNSHA) in humans. A severe deficiency was associated with hydrops fetalis, immediate neonatal death and neurological impairment [24]. Approximately 50 clinical cases of HNSHA associated with GPI deficiency have been reported [22]. By 2009, 29 mutations including 24 missense, 3 nonsense and 2 splice site were documented [24].

CLASSIFICATION AND STRUCTURE OF DIFFERENT TYPES OF PGI

Two different PGI superfamilies have been recently identified. A conventional PGI superfamily comprises a PGI family and PGI/PMI family. Two regions of conserved aa [DENS]-X-[LIVM]-G-G-R-[FY]-S-[LIVMT-X-[STA]-[PSAC]-LIVMA]-G- and [GS]-X-[LIVM]-[LIVMFYW]-X_4-[FY]-[DN]-Q-X-G-V-E-X_2-K were documented as specific motifs for the PGI superfamily [4]. The aa sequence S-Y-S-G-[NT]-T-[ESTIL]-E-T-[LIV] was described as a specific motif for PGI/PMI. PGIs contain a Lys or an Arg at 2nd position of this pattern [16].

PGI family has been divided into three major subfamilies: I – eukaryotic, II – cyanobacterial/chloroplasts and III – bacterial. Subfamily I also includes several eubacterial clades: *Zymonas mobilis*, *Xanthomonas campestris*, *E. coli* and *Haemophilus influenzae*. PGI subfamily II comprises phosphoglucose isomerases from chloroplasts, cyanobacteria and amitochondriate protists. Interestingly, PGIs from *Methanococcus jannaschii*, *Halobacterium* NRC1 and *Haloarcula marismortui* (all three strains are euryarcheota) were included into III subfamilies of PGI [16].

Putative homologs of the bifunctional *Aeropyrum pernix* PGI/PMI were identified in the genoms of eight (hyper)thermophilic Archae and bacteria: *Pyrobaculum aerophilum*, *Thermoplasma acidophilum*, *Thermoplasma volcanium*, *Ferroplasma acidarmanus*, *Sulfolubus tokodaii*, *Sulfolubus solfaraticus, Aquifex aelolicus* and *Anaerocellum thermophilum* [16].

The second superfamily of PGI cupin (cPGI) was discovered in the euryarcheota species *Pyrococcus furious* (*P. furiosus*) [14, 41], *Thermococcus litoralis* [21], *Archaeoglobulus fulgidus* [15] and *Methanosarcinia mazei* [15]. This type of PGI was found in two strains of bacteria *Salmonella enterica* serovar Typhimurium and *Ensifer meliloti* [15]. The motifs $TX_3PX_3GXEX_3TXGHXHX_{6\text{-}11}EXY$ and PPX_3HX_3N were deduced as two specific motifs of the cPGI super family. Phylogenetic analysis suggested later gene transfer for the bacterial cPGIs from euryarchaeota [15]. Cupin-PGI represents a class of PGI, which are completely unrelated to the αβ sandwich fold (SIS domain) presented in the conventional PGIs [3, 7]. The polypeptide chain of cPGI from *P. furiosus* starts with a short β strand, which forms an additional strand on the cupin barrel of the adjacent monomer. The chain continues through α-helix into two β-sheets, containing three and six β-strands. The chain is completed by an α-helix and two β-strands, which pack against the three-stranded β-sheet, and lead to the C-terminus. The active site of this and other cPGIs lies in the cupin barrel adjacent to the metal-biding site [7]. The two monomers in dimer are essentially identical [35]. Table 1 presents the comparison of PGI properties from different organisms.

Table 1. Comparison of PGIs from various organisms

Organism			Temp. opt. (°C)	pH opt.	Molecular weight (kDa)		K_m (mM)			Ref.
					native	subunit	F6P	G6P	M6P	
PGI superfamily	PGI-family	*E. coli* (I)	ND	8.0	120 (α_2)	59	0.2	ND	-	32
		E. coli (II)	ND	8.0	230 (α_4)	59	0.2	ND	-	32
		Human	ND	7.5	126 (α_2)	63	0.037 ± 0.003	ND	-	24
	PGI/ PMI-family	*A. pernix*	> 98	7.6 ± 0.2	45 ± 5	36	0.44 ± 0.10 0.21 ± 0.05 (50°C)	3.5 ± 0.5	1.1 ± 0.3	16
Cupin- PGI superfamily		*P. furiosus*	90	7.0	49.3 (α_2)	23.5	0.71	1.57	-	41

ND, not determined

MECHANISMS OF THE ALDOSE-KETOSE ISOMERIZATION

cPGI from *P. furiosus* similar to conventional type of PGI showed the activity in the isomerization of F6P and G6P. A further study concerning the substrate specificity of pyrococcal cPGI indicated that this enzyme was able to isomerize non-fosforylated sugar as L-talose and D-ribulose. L-Talose was converted to L-tagatose and L-galactose with 80% and 5% conversion yield, respectively. Whereas D-ribulose was converted to D-ribose and D-arabinose with 53% and 8% conversion yield, respectively [40].

The kinetic analysis revealed that bifunctional PGI/PGM catalyzed reversible isomerization of not only G6P, but also epimeric mannose-6-phosphate (M6P) [16]. Interestingly, M6P is a classical inhibitor for PGI and cPGI [17, 41].

Catalyzed by PGIs isomerization is reaction in which a hydrogen atom is transferred between the C1 and C2 position of substrate. The second hydrogen, in the form of a proton, also moves between the O1 and O2 [35]. In the mechanism postulated for PGI, proton exchange between O1 and O2 is likely to be mediated by a water molecule without the participation of active side residue [2, 26]. The carbon-bound hydrogen can moved by one of two mechanisms, a hydride shift or a proton transfer *via* a *cis*-enediol mechanism (Fig. 1) [30, 35].

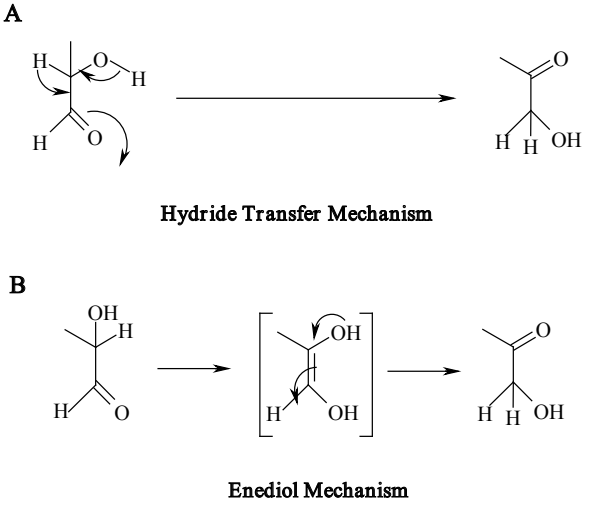

Fig. 1. The possible mechanisms for hydrogen transfer between the C1 and C2 position of substrate for phosphoglucose isomerase [after 30, 35]

Swan and his colleagues were favored a hydride transfer mechanism for cPGI from *P. furiosus* (Fig. 1A). A metal ion (Fe^{2+} *in vivo*) is rather involved in catalysis than perform a structural role. Pyrococcal PGI has a preference for straight chain substrates. This fact indicates the absence of any obvious enzymatic machinery indispensable for opening of sugar ring [35].

Berrisfold et al. [7] contrary to Swan and his co-workers postulated that the *cis*-enediol mechanism could also be employed in the *P. furiosus.* In this case, the function of metal in the *P. furiosus* PGI is to stabilize the developing charge on the intermediate.

Because of differences in opinion on mechanism of *P. furiosus* cPGI, further studies are required.

Conventional PGIs, which does not require a metal cation for activity, used acid-base mechanism with *cis*-enediol intermediate (Fig. 1B). These types of PGIs catalyze the isomerization with sugar ring opening. Lee and co-workers proposed His-388 as aa residue, which participate in the ring opening of cyclic substrates [23]. The inspection of the interaction of 5-phospho-D-arabinohydroxyamic acid (5 PAH) with rabbit PGI indicated that Glu-357 transfers a proton between C1 and C2. In the same study Arg-272 is proposed to help stabilize the *cis*-enediol(ate) intermediate [2].

POTENTIAL APPLICATIONS OF PGI AND ITS INHIBITORS

In the bacteria, EPS (compound of biofilm) provides a protective capsule against, e.g., dehydration, macrophages, antibiotics and other toxic compounds [37]. From this aspect, it is necessary to carry out a very intensive study upon enzymes (including PGI) involved in the biosynthesis EPS of pathogenic bacteria. On the other hand, bacterial EPS is widely used in many applications from food processing to pharmaceutical production and other industries [37]. An example of this type research is characterization of phosphoglucose isomerase from *Sphingomonas chungbukensis* DJ77 [36, 37].

Overexpression of AMF receptor is correlated with a poor prognosis of cancer [18]. The elevated AMF in serum or urine renders the protein tumor marker in gastrointestinal, kidney, breast, colorectal, and lung cancer. The presence of GPI in serum and urine is associated with cancer progression and indicates poor prognosis [5, 6, 11, 27].

The typical inhibitors as D-erythrose-4P, 6-phospho-D-gluconate, fructose-1-phosphate, fructose-1,6-bisphosphate and mannose-6-phosphate negatively affected various types of PGI [16, 17, 41]. Certainly, the PGI/PMM was not inhibited by mannose-6-P, which is a substrate for these type enzymes [16].

PGI are attractive targets for chemotherapeutic actions of antiparasites or antifungal drug. Firstly, parasites like *Trypanosoma brucei, Trypanosoma cruzi* and *Plasmodium falciparum* derive most their energy from glycolysis [17]. Certainly, the specific inhibition of this cycle in parasites, but not in human, is necessary. Secondly, several inhibitors were tested, which mimic the transition state intermediates in PGI and hexose phosphate domain of D-glucosamine synthase (EC 2.6.1.16) [17, 26, 43]. 5-phosho-D-arabinoate (5PA), 6-phospho-D-gluconate and 5-PAH have been reported as strong inhibitors of PGI, which mimics the transition state intermediates [17]. From these compounds, 5-PAH is the best reported inhibitor of PGI with a K_i of 2 x 10^{-7}M (pH 8.0) [2, 17]. Wojciechowski and co-workers proposed D-glucosamine synthase (L-glutamine: D-F6P amidotransferase (hexose isomerizing) as a new target for antifungal drugs [26, 43]. In fungi and bacteria, differently in mammal cells, short-time depletion of this enzyme is lethal [26, 43]. From the tested compounds 2-amino-hexitol phosphates, 2-amino-2-deoxy-D-mannitol-6-phosphate (ADMP) was a better inhibitor for *Candida albicans* D-glucosamine synthase than 2-amino-2-deoxy-D-glucitol-6-phosphate (ADPG). These two investigated compounds were not in the same extent effective against *Saccharomyces cerevisiae* PGI [26, 43].

CONCLUSIONS

Jeffery indicated that treatment that corrects only one function of "moonlighting" protein might not be sufficient to treat the disease. On the other hand, adjusting the level of one activity of the enzyme might be indispensable, because adjusting both might result in side effects [20]. From these aspects, further characterization of PGI as an example of "old proteins which were learning new tricks" [20] is necessary.

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SUMMARY

In this article, the biological functions, classification, structures, mechanisms of reaction and potential applications of the phosphoglucose isomerase and its inhibitors were discussed. The knowledge of various biological processes in which PGI is involved can be very helpful in the treatment of cancer, bacteriosis, mycosis and parasitosis.

Keywords: isomerase, biological function, classification structures, mechanism of reaction

STRESZCZENIE

W artykule przeglądowym przedstawiono biologiczne funkcje, klasyfikację, struktury, mechanizmy reakcji i potencjalne zastosowanie izomerazy fosfoglukozowej i inhibitorów tego enzymu. Wiedza o różnych biologicznych procesach, w których zaangażowana jest PGI, może być bardzo pomocna w leczeniu nowotworów, bakterioz, grzybic i parazytoz.

Słowa kluczowe: izmoeraza fosfoglukozowa, biologiczne funkcje, klasyfikacja struktury, mechanizmy relacji