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Bioinformatic Analysis of Phosphoglucomutase (PGM2) from Different Species of Plasmodia Using Computational Tools

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Abstract

In this study, PGM2 enzyme from different malaria parasite *Plasmodium* species was analyzed and presented in this communication. The composition of leucine, lysine and asparagines were the highest while lowest concentrations of tryptophan and histidine residues were noticed when compared to other amino acids. The pI value of *P. vinckei* PGM2 was 8.59 while the lowest pI of 5.91 was shown by *P. vivax* PGM2. The instability index of all the enzymes is variable, but for all of them it was less than 40, which indicates that all of them are stable. The aliphatic index was found to span within a range of 83 to 86. Secondary structural analysis of the enzymes showed the pre-dominance of Alpha helix followed by random coils for all the mutases except *P. vivax*, *P. inui*, *P. Knowlesi* and *P. Fragile* PGM2 enzyme. The significance of the above results is discussed in the light of existing literature.

Keywords

PGM2, Phosphoglucomutase, *Plasmodium spp.*, Bioinformatics, Secondary Structure

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1. Introduction

A mutase is an enzyme of the isomerase class that catalyzes the shifting of a functional group from one position to another within the same molecule. Phosphoglucomutase (PGM) is a key enzyme in carbohydrate metabolic pathway and is responsible for the conversion of D-glucose1-phosphate into D-glucose6-phosphate, which is then converted into uridine diphosphate–glucose. PGM participates in both the breakdown and synthesis of glucose (Dai *et al*., 1992). The enzyme (PGM) reversibly catalyses the transfer of phosphate between the C6 and C1 hydroxyl groups of glucose. PGM thus, plays a pivotal role in the synthesis and utilization of glycogen and is present in all organisms. In humans, there are three well-described isozymes, PGM1, PGM2, and PGM3 (Whitehouse *et al*., 1998).

PGM1 were detected in *P. Falciparum* resistant malaria as phenotype of unknown relevance to protection against falciparum malaria (Bayoumi *et al*., 1986). PGM has been used as successful genetic marker for genotyping *Leishmania tropica* from clinical samples, and thus saves the effort of culturing or multilocus enzyme electrophoresis methods (Azmi *et al*., 2013). Some parasitic protozoa such as *Trypanosoma brucei* lack the PGM enzyme (Bandini *et al*., 2012). *Trypanosoma cruzi* relies on highly galactosylated molecules as virulence factors and the enzymes involved in sugar biosynthesis are potential therapeutic targets. The synthesis of UDP-galactose in *T. Cruzi* requires the activity of phosphoglucomutase (PGM) (Penha *et al*., 2009). Several enzymes that participate in carbohydrate metabolism in trypanosomes are located in the glycosomes (Penha *et al*., 2009).

The Plasmodia PGM2 have been considered previously as

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virulent factor and thus potential drug target (Olliaro and Yuthavong, 1999), however, the functional study is hampered by the difficulty of either culturing of Plasmodia species or the purification and activation challenge. Therefore, *in silico* study on the Plasmodia PGM2 enzyme from different species or hosts, will asset in revealing the similarity and/or dissimilarity in the structure and hence the function of the enzyme. In the present study, bioinformatic analysis of PGM enzymes from the human (falciparum, vivax), rodent (berghei, chabaudi, vinckei, yoelii) primate (fragile, inui, knowlesi, reichenowi), malaria parasite *Plasmodium sp*. is communicated.

2. Materials and Methods

UniProtKB/Swiss-Prot, a protein sequence database, was used to retrieve the complete sequences of all the Plasmodia PGM2 enzymes used in this study (Bairoch and Apweiler, 2000). The *P. falciparum* PGM2 sequence was obtained from PlasmoDB (Bahl *et al*., 2002). Blast search was performed for some of the Plasmodia PGM2 sequences (Altschul *et al*., 1990; Altschul *et al*., 1997). These sequences were used for further analysis. The computation of various physical and chemical parameters was performed using ExPASy's ProtParam tool (Gasteiger *et al*., 2001). The SOPM Atool (Self-Optimized Prediction Method with Alignment) server was used to characterize the secondary structural features (Geourjon and Deleage, 1995). The SOSUI server was used to predict the transmembrane regions which were further classified as membrane bound and soluble proteins (Gomi *et al*., 2004; Pagni *et al*., 2007).

3. Results and Discussion

It is well known that, after glycogen phosphorylase catalyzes the phosphorolytic cleavage of a glucosyl residue from the glycogen polymer, the freed glucose will have a phosphate group on its 1-carbon. This glucose1-phosphate molecule is not itself a useful metabolic intermediate, but PGM catalyzes the conversion of this glucose1-phosphate to glucose 6-phosphate (Najjar and Pullman, 1954; Rhyu *et al*., 1984). Glucose 6-phosphate's metabolic fate depends on the needs of the cell at the time it is generated. If the cell is low on energy, then glucose 6-phosphate will travel down the glycolytic pathway, eventually yielding two molecules of ATP. If the cell is in need of biosynthetic intermediates, glucose 6-phosphate will enter the pentose phosphate pathway, where it will undergo a series of reactions to yield riboses and/or NADPH, depending on cellular conditions (Brown, 1986). However, the glucose's metabolic fate has been investigated in the *P. falciparum* (Lian *et al*., 2009).

The PGM is also the pivotal enzyme that catalyzes the reversible interconversion of glucose-6-phosphate into glucose-1-phosphate, an intermediate required for the synthesis of UDP-Gal*p*. Accordingly, the activity of PGM should be essential for the biosynthesis of UDP-Gal*p* in *Plasmodium spp*.

In most organisms, the synthesis of sugar nucleotides occurs in the cytoplasm and the precursors are subsequently transported to the Golgi to be incorporated in sugar moieties (Hirschberg *et al*., 1998; Berninsome and Hirschberg, 2000). Previous studies have demonstrated the presence of a microsome-bound form of cytosolic phosphoglucomutase in rat (Mithieux *et al*., 1995). Using a phylogenetic strategy, 47 highly divergent prokaryotic and eukaryotic PGM-like sequences were identified from the database in previous study (Whitehouse *et al*., 1998). Although overall amino acid identity fell below 20%, the relative order, position, and sequence of three structural motifs, the active site and the magnesium - and sugar-binding sites, were conserved in all 47 sequences (Whitehouse *et al*., 1998).

In this study, the PGM2 enzymes families from Plasmodium genus were analyzed and the results are presented. Comparative analysis of the PGM2 enzymes may give new inputs as to which groups of the Plasmodia PGM2 are more suitable for functional investigation as anti-malarial drug target.

Table 1 shows that the amino acid composition of 10 different PGM2 enzymes of *Plasmodium* species found in biological databases. The composition of leucine, lysine and asparagines was the highest while lowest concentrations of tryptophan and histidine residues were noticed when compared to other amino acids. The number of negative charged residues is more than the positively charged residues (Table 2). The molecular weight of *P. yoelii* PGM2 enzyme was the highest while *P. knowlesi* PGM2 had the lowest molecular weight. The pI value of *P. vinckei* PGM2 was 8.59 while the lowest pI of 5.91 was noticed in *P. vivax* PGM2. The instability index of all the PGM2 enzymes is variable but for all of them it was less than 40 showing that all of them are stable. The aliphatic index showing the relative volume of protein occupied by aliphatic side chains was found to span within a range of 83 to 86. From Table 3, secondary structural analysis of the Plasmodia PGM2 transferases showed the pre-dominance of alpha helix followed by random coils for all of the mutases except *P. vivax*, *P. inui*, *P. knowlesi* and *P. fragile* PGM2 enzyme. The SOSUI server analysis (Table 4) has shown that all of the Plasmodia PGM2 transferase enzymes are soluble proteins. These *in silico* findings could possibly be valuable for working on properties of Plasmodia PGM2 enzymes in solution.

Table 1. Amino acid composition of different PGM2 transferases from *Plasmodium* species.

Species	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
falciparum	5.2	2.7	7.9	5.2	2.9	2.7	6.6	5.2	2.2	7.6	8.6	8.4	2.9	5.2	3.0	5.4	5.2	1.0	6.2	5.7
vivax	5.4	3.9	6.1	5.6	2.5	2.7	6.1	6.2	2.0	5.6	8.6	6.4	2.9	4.9	3.4	7.1	5.7	1.0	6.4	7.6
inui	5.4	3.9	6.6	5.1	2.5	2.4	6.1	6.2	2.4	6.2	8.8	6.9	2.5	4.9	3.4	7.1	5.6	1.0	6.1	7.1
berghei	3.0	4.8	6.5	6.5	2.6	0.9	6.1	6.5	2.2	7.4	9.5	7.4	1.7	6.5	3.5	8.2	5.2	1.0	6.1	5.2
yoelii	4.7	2.9	8.8	5.7	2.5	2.9	6.2	5.2	1.7	7.3	8.8	8.3	2.5	5.1	3.4	5.7	4.9	1.0	6.7	5.7
knowlesi	5.7	3.5	6.6	5.4	2.5	2.5	5.9	6.4	1.7	6.1	8.8	7.3	2.7	4.9	3.2	6.4	5.9	1.0	6.6	6.9
chabaudi	5.2	2.7	7.1	6.9	2.7	2.7	5.1	5.4	2.2	7.6	8.3	10.3	2.2	4.9	3.5	6.6	4.6	1.0	5.6	5.6
vinckei	5.6	3.2	6.7	6.1	2.7	2.7	5.2	5.2	1.7	7.8	8.6	9.6	2.4	5.1	3.5	6.2	5.2	1.0	6.2	5.2
fragile	6.2	3.5	6.4	5.4	2.5	2.9	5.7	5.9	2.0	6.2	8.8	7.1	2.7	4.9	3.5	6.6	5.4	1.0	6.4	6.7
reichenowi	5.2	2.9	7.9	5.2	2.7	2.7	6.6	5.4	2.2	7.4	8.8	8.3	2.9	5.2	3.0	5.4	5.2	1.0	6.2	5.7

Table 2. Physicochemical characteristics of Plasmodial PGM2 transferases.

Name of species	No of amino acids	Molecular weight	pI	-ve charged residues	+ve charged residues	Instability index	Aliphatic index	gravy
falciparum	593	68300.4	6.39	70	66	32.41	84.99	-0.269
vivax	593	67482.9	5.91	69	61	34.56	82.65	-0.224
inui	593	67461.1	6.71	66	64	36.43	84.47	-0.228
berghei	593	68160.8	6.30	69	65	34.02	85.97	-0.316
yoelii	593	68416.1	6.13	71	66	33.98	83.83	-0.345
knowlesi	593	67450.1	6.40	67	64	33.27	83.66	-0.223
chabaudi	593	67781.7	8.36	71	77	29.59	83.19	-0.369
vinckei	593	68039.3	8.59	67	76	32.87	84.52	-0.304
fragile	593	67480.2	6.48	66	63	36.75	84.33	-0.217
reichenowi	593	68282.3	6.39	70	66	34.04	84.99	-0.276

Table 3. Secondary structure of Plasmodia PGM2 transferases.

Species	Alpha helix	3_{10} helix	Pi helix	Beta bridge	Extended strand	Beta turn	Bend region	Random coil	Ambiguous state	Other states
falciparum	41.65	0.00	0.00	0.00	21.42	0.00	0.00	36.93	0.00	0.00
vivax	34.74	0.00	0.00	0.00	25.46	0.00	0.00	39.80	0.00	0.00
inui	35.08	0.00	0.00	0.00	26.48	0.00	0.00	38.45	0.00	0.00
berghei	40.98	0.00	0.00	0.00	18.55	0.00	0.00	40.47	0.00	0.00
yoelii	41.32	0.00	0.00	0.00	18.38	0.00	0.00	40.30	0.00	0.00
knowlesi	32.21	0.00	0.00	0.00	26.98	0.00	0.00	40.81	0.00	0.00
chabaudi	42.16	0.00	0.00	0.00	18.38	0.00	0.00	39.46	0.00	0.00
vinckei	46.21	0.00	0.00	0.00	17.37	0.00	0.00	36.42	0.00	0.00
fragile	36.76	0.00	0.00	0.00	23.78	0.00	0.00	39.46	0.00	0.00
reichenowi	41.65	0.00	0.00	0.00	20.91	0.00	0.00	37.44	0.00	0.00

Table 4. Prediction of transmembrane regions of the Plasmodia PGM2 transferases.

Species	N terminal	Transmembrane region	C terminal	Type	Length	Protein
falciparum	0	0	0	0	0	soluble
vivax	0	0	0	0	0	soluble
inui	0	0	0	0	0	soluble
berghei	0	0	0	0	0	soluble
yoelii	0	0	0	0	0	soluble
knowlesi	0	0	0	0	0	soluble
chabaudi	0	0	0	0	0	soluble
vinckei	0	0	0	0	0	soluble
fragile	0	0	0	0	0	soluble
reichenowi	0	0	0	0	0	soluble

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