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Bioinformatical Analysis of HGPRT Transferase from Different Malaria Parasite Plasmodium spp. Using Computational Tools

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ABSTRACT

In this study, HGPRT transferases from different malaria parasite *Plasmodium* species was analyzed and presented in this communication. The composition of leucine, lysine and Isoleucine were the highest while lowest concentrations of tryptophan and glutamine residues were noticed when compared to other amino acids. pI value of *P. reichenowi* HGPRT was 7.59 while the lowest pI of 6.22 was shown by *P. chabaudi* HGPRT. The instability index of all the transferases is varied, 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 97. Secondary structural analysis of the transferases showed the predominance of random coils followed by extended strands for all the transferases except *P. falciparum*, *P. Knowlesi* and *P. reichenowi* HGPRT transferase. The significance of the above results is discussed in the light of existing literature.

Keywords: HGPRT – Phosphoribosyltransferase - Plasmodium spp. - Bioinformatics – Secondary structure

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INTRODUCTION

Purine nucleotide requirements of all organisms are met either through the *de novo* synthesis of purines from amino acids, ribose, carbon dioxicide and formate or by the salvage pathways or by the concomitant use of both. In the salvage pathway, hypoxanthine, guanine and adenine released by degradation of nucleic acids are converted back into nucleotides (Reyes *et al*., 1982).

The malaria parasite, like most other parasitic protozoans, cannot synthesize purines *de novo* and thus, relies solely on salvage pathways for its purine nucleotide requirements (Sherman, 1979). This dependence of *Plasmodium* on the purine salvage pathway for its growth and development makes the enzymes of this pathway candidate targets for drug development (Ullman and Carter, 1995). Several studies have investigated the pathways for the transport of purine nucleosides into the parasite. For example, 2'- deoxycoformycin, an inhibitor of adenosine deaminase, produces a dramatic reduction in parasitemia in *P. knowlesi* infected rhesus monkeys, indicating that a block in hypoxanthine production is detrimental for parasite growth (Webster *et al*., 1984). Addition of xanthine oxidase, which depletes hypoxanthine in the culture medium, also leads to the death of the parasite, providing additional evidence that hypoxanthine is the main source of purine to the intraerythrocytic parasite (Berman *et al*., 1991).

The parasite enzyme has been purified from *in vivo* parasite cultures and its additional specificity for xanthine documented (Queen *et al*., 1988). The gene encoding the parasite HGXPRT has been cloned and expressed in *E. coli* (Vasanthakumar *et al*., 1990). The gene functionally complements HGXPRT deficiency in the *E. coli* validating that a

functional enzyme was produced in the strain (Shahabuddin and Scaife, 1990). However, the recombinant *P. falciparum* HGXPRT was inactive and, therefore, functional studies with the enzyme were not possible (Sujay Subbayya and Balaram, 2000).

The Plasmodia HGPRT is a 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 HGPRT from different species or hosts, will asset in revealing the similarity or differences in the structure and hence the function of the enzyme. In the present study, bioinformatic analysis of HGPRT transferases from the human (falciparum, vivax), rodent (berghei, chabaudi, vinckei, yoelii) primate (fragile, inui, knowlesi, reichenowi), malaria parasite *Plasmodium sp*. is communicated.

MATERIALS AND METHODS

UniProtKB/Swiss-Prot, a protein sequence database, was used to retrieve the complete sequences of all the Plasmodia HGPRT transferases (Bairoch and Apweiler, 2000). *P. falciparum* HGPRT sequence was obtained from PlasmoDB (Bahl *et al*., 2002). Blast search was performed for some Plasmodia HGPRT 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 done using ExPASy's ProtParam tool (Gasteiger *et al*., 2001). SOPMA tool (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 that were further classified as membrane-bound and soluble proteins (Pagni *et al*., 2007).

RESULTS AND DISCUSSION

Phosphoribosyltransferases (PRT) are enzymes that catalyze the synthesis of β-n-5'-monophosphates from phosphoribosylpyrophosphate (PRPP) and an enzyme specific amine. Some of PRT's are involved in the biosynthesis of purine, pyrimidine, and pyridine nucleotides, or in the salvage of purines and pyrimidines. These enzymes are:

(a). Adenine phosphoribosyltransferase (EC 2.4.2.7) (APRT), which is involved in purine salvage.

(b). Hypoxanthine-guanine or hypoxanthine phosphoribosyltransferase (EC 2.4.2.8) (HGPRT or HPRT), which are involved in purine salvage.

(c). Orotate phosphoribosyltransferase (EC 2.4.2.10) (OPRT), which is involved in pyrimidine biosynthesis.

(d). Amido phosphoribosyltransferase (EC 2.4.2.14), which is involved in purine biosynthesis.

(e). Xanthine-guanine phosphoribosyltransferase (EC 2.4.2.22) (XGPRT), which is involved in purine salvage.

In the sequence of all these enzymes, there is a small conserved region that may be involved in the enzymatic activity and be part of the PRPP binding site (Hershey and Taylor, 1986).

Phosphoribosyltransferases (PRTases) constitute a group of enzymes that participate in the biosynthesis of pyrimidine, purine, and pyridine nucleotides, as well as the amino acids histidine and tryptophan (Musick, 1981). There are at least ten different PRTases known which transfer phosphor-ribose on to different substrates. The nucleotide formation chemistry involves inversion of stereochemistry at the numeric carbon of the ribose C1 and has been proposed to proceed via a S_N1 or S_N2 mechanism (Craig and Eakin, 2000). Structurally the PRTases fall into two groups: type I and type II. The type I fold seen in the structures of orotate PRtase, HGPRTase, adenine PRTase, uracil PRTase and glutamine phosphoribosyl aminotransferase (GPATase) consists of five parallel β strands surrounded by 3 -4 α helices. These PRTases contain a conserved motif of 13 residues, involved in the binding of the ribose phosphate moiety of the PRTase fold (Craig and Eakin, 2000).

Type II PRTases have been studied less extensively and the only structures available are that of quinolinic acid phosphoribosyl transferase (Sharma *et al*., 1998). The type II PRTase fold consists of an N-terminal four-stranded open face β-sandwich domain and a C-terminal α/β – barrel domain. Despite their distinct structural folds, type I and II PRTases carryout very similar enzymatic reactions involving the domain of a ribose 5- phosphate unit from PRPP to a nitrogenous base to form the nucleotide and pyrophosphate as products. The RPTases are, therefore, an example of convergent evolution towards a similar enzymatic activity (Eads *et al*., 1997 and Sharma *et al*., 1998). The HGPRT transferases from *Plasmodium* genus family were analyzed, and the results are presented. Comparative analysis of the HGPRT transferases may give new inputs as to which groups of the Plasmodia PRTeases are suitable for functional investigation as anti-malarial drug target.

Table 1 shows that the amino acid composition of ten different HGPRT transferases of *Plasmodium* species found in biological databases. The composition of leucine, lysine and Isoleucine was the highest while lowest concentrations of tryptophan and glutamine residues were seen when compared to other amino acids. The number of negatively charged residues are more than the positively charged residues (**Table 2**). The molecular weight of *P. inui* HGPRT transferase was the highest while *P. falciparum* HGPRT and *P. reichenowi* HGPRT had the lowest molecular weight. The pI value of *P. reichenowi* HGPRT was 7.59 while the lowest pI of 6.22 was shown by *P. chabaudi* HGPRT. The instability index of all the transferases varied but for all of them it was less than 40 showing that all of them are stable. An aliphatic index showing the relative volume of protein occupied by aliphatic side chains was found to span within a range of 83 to 97. From **Table 3**, Secondary structural analysis of the Plasmodia HGPRT transferases showed the pre-dominance of random coils followed by extended strands for all the transferases except *P. falciparum*, *P. Knowlesi*, and *P. reichenowi* HGPRT transferase. SOSUI server analysis (**Table 4**) has shown that all the Plasmodia HGPRT transferases were soluble proteins and only *P. inui* HGPRT is membrane bound protein. These *in silico* findings can be used for working on properties of Plasmodia HGPRT transferases in solution.

Table 1: Amino acid composition of different HGPRT 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	4.3	2.6	5.6	7.4	3.0	0.4	5.6	6.1	3.5	6.9	10.0	10.4	0.9	6.5	3.9	5.2	4.3	0.4	5.2	7.8
vivax	3.9	3.9	5.6	8.2	2.6	0.9	6.0	4.7	3.0	9.0	10.3	9.9	0.4	6.0	4.3	4.7	4.3	0.4	5.6	6.4
inui	2.5	4.2	4.6	7.9	2.9	0.8	5.8	4.6	2.9	9.2	10.0	9.2	0.4	6.7	4.2	7.1	5.0	0.4	5.4	6.2
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	0.4	6.1	5.2
yoelii	2.6	4.8	6.1	6.5	2.6	0.9	6.1	7.8	2.2	8.2	8.2	7.4	1.7	6.5	3.9	7.4	4.8	0.4	6.1	6.1
knowlesi	3.9	4.7	5.6	7.7	2.6	0.0	6.4	5.2	3.0	8.6	10.3	9.4	0.9	6.0	3.4	6.0	4.3	0.4	5.6	6.0
chabaudi	3.5	4.3	5.6	6.9	2.6	1.3	6.1	6.5	2.2	7.4	10.0	7.8	1.7	6.5	3.9	6.5	5.6	0.4	6.1	5.2
vinckei	3.0	4.8	5.6	6.9	2.6	1.7	5.6	6.9	2.2	6.9	10.0	7.4	1.3	6.5	3.9	7.4	4.8	0.4	6.1	6.1
fragile	3.9	4.7	6.0	7.3	2.6	0.0	6.9	4.7	3.0	8.6	9.4	9.4	1.3	6.0	3.9	6.0	3.9	0.4	5.6	6.4
reichenowi	4.3	2.6	5.2	7.4	3.0	0.4	5.6	6.1	3.5	6.5	10.0	10.8	0.9	6.5	3.9	5.2	4.3	0.4	5.2	8.2

Table 2: Physico chemical characteristics of Plasmodial HGPRT transferases

Name of species	No of amino acids	Molecular weight	pI	-ve charged residues	+ve charged residues	Instability index	Aliphatic index	gravy
falciparum	231	26348.4	7.06	30	30	30.64	92.77	-0.160
vivax	233	26970.1	6.66	33	32	27.34	97.85	-0.230
inui	240	27792.0	6.65	33	32	33.38	95.38	-0.193
berghei	231	26525.2	6.52	29	28	27.80	83.94	-0.288
yoelii	231	26418.1	6.52	29	28	22.43	84.33	-0.257
knowlesi	233	26955.0	7.04	33	33	35.80	94.94	-0.249
chabaudi	231	26546.4	6.22	30	28	24.50	86.06	-0.258
vinckei	231	26485.2	6.52	29	28	26.70	86.45	-0.262
fragile	233	27026.1	7.04	33	33	34.36	92.83	-0.273
reichenowi	231	26348.4	7.59	30	31	29.68	92.34	-0.163

Table 3: Secondary structure of Plasmodia HGPRT transferases

Species	Alpha helix	3_{10} helix	Pi helix	Beta bridge	Extended strand	Beta turn	Bend region	Random coil	Ambiguous state	Other states
falciparum	32.90	0.00	0.00	0.00	25.54	0.00	0.00	41.56	0.00	0.00
vivax	22.32	0.00	0.00	0.00	30.47	0.00	0.00	47.21	0.00	0.00
inui	18.75	0.00	0.00	0.00	33.75	0.00	0.00	47.50	0.00	0.00
berghei	20.78	0.00	0.00	0.00	33.33	0.00	0.00	45.89	0.00	0.00

yoelii	19.48	0.00	0.00	0.00	33.77	0.00	0.00	46.75	0.00	0.00
knowlesi	29.61	0.00	0.00	0.00	25.32	0.00	0.00	45.06	0.00	0.00
chabaudi	22.51	0.00	0.00	0.00	30.30	0.00	0.00	47.19	0.00	0.00
vinckei	22.08	0.00	0.00	0.00	29.44	0.00	0.00	48.48	0.00	0.00
fragile	22.75	0.00	0.00	0.00	29.18	0.00	0.00	48.07	0.00	0.00
reichenowi	32.03	0.00	0.00	0.00	27.27	0.00	0.00	40.69	0.00	0.00

Table 4: Prediction of transmembrane regions of the Plasmodia HGPRT transferases

No.	N terminal	Transmembrane region	C terminal	Type	Length	Protein
falciparum	0	0	0	0	0	soluble
vivax	0	0	0	0	0	soluble
inui	1	MTPQNVNIFFLCSPGAGESA	20	Signal Peptide	20	Membrane bound
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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REFERENCES

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. (1990) Basic local alignment search tool. J. Mol. Biol. 215: 403-10.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25: 3389-402.

Bahl A, Brunk B, Coppel RL, Crabtree J, Diskin SJ, Fraunholz MJ, Grant GR, Gupta D, Huestis RL, Kissinger JC, Labo P, Li L, McWeeney SK, Milgram AJ, Roos DS, Schug J, Stoeckert CJ Jr. (2002) Plasmo DB: the Plasmodium genome resource. An integrated database providing tools for accessing, analyzing and mapping expression and sequence data (both finished and unfinished). Nucleic Acids Res. 2002 Jan 1; 30(1): 87-90.

Bairoch A. and R. Apweiler R. (2000). "The SWISS-PROT protein sequence database and its supplement TrEMBL in 2000," Nucl. Acids Res., vol. 28, pp. 45–48, 2000.

Berman PA, Human L, Freese JA. (1991) Xanthine oxidase inhibits growth of *Plasmodium falciparum* in human erythrocytes in vitro. J Clin Invest. 1991 Dec; 88(6): 1848-55.

Combet C., Blanchet C., Geourjon C. and Deléage G. (2000). NPS@: Network Protein Sequence Analysis. TIBS 2000 March Vol. 25, No 3 [291]:147-150.

Craig SP 3rd and Eakin AE. (2000) Purine phosphoribosyl transferases. J Biol Chem. 2000 Jul 7; 275(27): 20231-4.

Eads JC, Ozturk D, Wexler TB, Grubmeyer C, Sacchettini JC. (1997) A new function for a common fold: the crystal structure of quinolinic acid phosphoribosyltransferase. Structure. 1997 Jan 15; 5(1): 47-58.

Gasteiger E., Jung E. Bairoch A. (2001). "SWISS-PROT: Connecting biological knowledge via a protein database," Curr. Issues Mol. Biol., vol. 3, pp. 47–55, 2001.

Geourjon C. and Deleage G. (1995). "SOPMA: Significant Improvements in protein secondary structure prediction by consensus prediction from multiple alignments," Comput. Appl. Biosci., vol. 11, pp. 681–684, 1995.

Gomi M., Sonoyama M., and Mitaku S. (2004). High performance system for signal peptide prediction: SOSUI signal Chem-Bio Info. J., 4: 142-147.

Hershey H.V., Taylor M.W. Nucleotide sequence and deduced amino acid sequence of *Escherichia coli* adeninephosphoribosyl transferase and comparison with other analogous enzymes. Gene 43:287-293 (1986).

Musick WD. (1981) Structural features of the phosphoribosyl transferases and their relationship to the human deficiency disorders of purine and pyrimidine metabolism. CRC Crit Rev Biochem. 1981; 11(1):1-34.

Olliaro PL & Yuthavong Y (1999) An overview of chemotherapeutic targets for antimalarial drug discovery. Pharmacol Ther 81: 91–110.

Pagni M. Ioannidis V. Cerutti L. Zahn-Zabal M. Jongeneel C. Jo¨rg H. Olivier M. Dmitri K. Laurent F. (2007) "MyHits: Improvements to an interactive resource for analyzing protein sequences," Nucleic Acids Res., vol. 35, pp. W433–W437, 2007.

Queen SA, Vander Jagt DL, Reyes P. (1988) Properties and substrate specificity of a purine phosphoribosyl transferase from the human malaria parasite, *Plasmodium falciparum*. Mol Biochem Parasitol. 1988 Aug; 30 (2): 123-33.

Reyes P, Rathod PK, Sanchez DJ, Mrema JE, Rieckmann KH, Heidrich HG. (1982) Enzymes of purine and pyrimidine metabolism from the human malaria parasite, *Plasmodium falciparum*. Mol Biochem Parasitol. 1982 May;5(5):275-90.

Shahabuddin M, Scaife J. (1990) The gene for hypoxanthine phosphoribosyl transferase of *Plasmodium falciparum* complements a bacterial HPT mutation. Mol Biochem Parasitol. 1990 Jun; 41(2): 281-8.

Sharma V, Grubmeyer C, Sacchettini JC. (1998) Crystal structure of quinolinic acid phosphoribosyltransferase from Mycobacterium tuberculosis: a potential TB drug target. Structure. 1998 Dec 15; 6(12): 1587-99.

Sherman I. W. (1979) Biochemistry of Plasmodium (malarial parasites). Microbiol Rev. 1979 Dec; 43(4): 453-95.

Sujay Subbayya IN and Balaram H (2000). Evidence for multiple active states of *Plasmodium falciparum* hypoxanthine-guanine-xanthine phosphoribosyl transferase. Biochem Biophys Res Commun 279: 433–437.

Ullman, B., Carter, D. (1995) Hypoxanthine-guanine phosphoribosyltransferase as a therapeutic target in protozoal infections. Infect Agents Dis. 1995 Mar; 4(1): 29-40.

Vasanthakumar G, Davis RL Jr, Sullivan MA, Donahue JP. (1990) Cloning and expression in *Escherichia coli* of a hypoxanthine-guanine phosphoribosyl transferase-encoding cDNA from *Plasmodium falciparum*. Gene. 1990 Jul 2; 91(1): 63-9.

Webster HK, Wiesmann WP, Pavia CS. (1984) Adenosine deaminase in malaria infection: effect of 2'-deoxycoformycin in vivo. Adv Exp Med Biol. 1984; 165 Pt A: 225-9.

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