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Research article

ROLE OF CARBON IN CRYSTAL STRUCTURES OF WILD-TYPE AND MUTATED FORM OF DIHYDROFOLATE REDUCTASE-THYMIDYLATE SYNTHASE OF *PLASMODIUM FALCIPARUM*

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ABSTRACT: *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) is an important target of antimalarial drugs. A characteristic set of mutations in this bifunctional enzymatic protein leads to reduced competitive drug binding at the enzyme's active site. The carbon content distribution study at mutational sites and along the amino acid sequence of this important protein was carried out using carbon analysis tool CARBANA. The mutational sites at residues 16, 51, 59, 108, and 164 were investigated. The study reveals that the carbon content and distribution of A16V and S108N mutants is shifting towards a normal distribution of the carbon content which is symmetrical about 0.3145, conforming to the value for stable and ordered protein. The study also reveals that carbon distribution of PfDHFR-TS mutant protein is maintained at 31.45% of carbon all along the sequence. The hydrophobicity of the entire sequences also balances quite well at the optimum position and carbon is the only element contributing towards this stability. Thus, the study of carbon distribution in mutations of PfDHFR-TS is the most significant step towards understanding the biological features which can provide possible approaches for the design of new drugs to overcome antifolate resistance.

Key words: *Plasmodium falciparum,* Mutation, Enzyme, Dihydrofolate reductase, Thymidylate Synthase, carbon distribution.

INTRODUCTION

Plasmodium falciparum, the most deadly malaria pathogen species is responsible for many deaths resulting from malaria disease. The disease is thought to have been the greatest selective pressure on the human genome in recent history (Kwiatkowski, 2005). The World Health Organization has estimated that malaria annually causes 250 million cases of fever (WHO, 2008). In 2010, the organization estimated that 655,000 people died from the disease of which 91% were in Africa with the majority of cases occurring in children under 5 years old (WHO, 2011). However, a 2012 meta-study on global malaria mortality estimated that deaths are significantly higher, with 1,238,000 people dying from malaria in 2010 (Murray *et al.,* 2012).

Plasmodium falciparum Dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) is a bifunctional enzyme in the folate biosynthesis pathway, being an important target of traditional antimalarial drugs such as pyrimethamine and cycloguanil (Yuthavong, 2002). DHFR catalyses the production of tetrahydrofolate from dihydrofolate while TS is in charge of transferring a methyl-group from N5, N10-methylene-tetrahydrofolate to dUMP thereby generating dTMP and tetrahydrofolate. Mutations in PfDHFR gene, associated with the amino acid substitution at residues 16, 51, 59, 108, and 164 are known to augment different levels of resistance to antifolate drugs such as pyrimethamine and cycloguanil (Yuvaniyama *et al.,* 2003; Dasgupta *et al.,* 2009; Lozovsky *et al.,* 2009).

The function of a protein mainly depends on its structure and stability. Although various studies have been carried out to reveal the stability of PfDHFR-TS protein upon amino acid substitutions due to mutations (Chusacultanachai *et al.,* 2002; Gromiha *et al.,* 2002; Gromiha, 2003; Lozovsky *et al.,* 2009; Tipsuwan *et al.*, 2011), to date no study has been done to evaluate the role of carbon in mutations of this important protein of malaria parasite.

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Carbon is the only element that contributes towards the dominant force, hydrophobic interaction in proteins in response to proteins' stability and activity. It is reported that proteins prefers to have 31.45% carbon for its stability (Rajasekaran and Vijayasarathy, 2011). Furthermore, mutational studies are helpful to understand the stability of proteins, to delineate the important residues for protein function, activity and drug design. The carbon distribution along the pfDHFR-TS mutants at residues 16, 51, 59, 108, and 164 are investigated and reported.

MATERIALS AND METHODS

The protein sequences of wild-type and mutated *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) were collected from Protein Data Bank (PDB) (http://www.rcsb.org/pdb). PDB accession numbers were 1J3I and 1J3K for wild-type and mutant proteins respectively based on protein previously reported (Yuvaniyama *et al.,* 2003). Further details of the mutation sites were obtained from Protein Mutant Database (PMD) (http://pmd.ddbj.nig.ac.jp/~pmd/pmd.html) providing information of amino acid mutations at specific positions of the proteins. To study the mutational sites of the protein sequences CARD program (Akila *et al.,* 2012) was used. An outer length of 135 and inner length of 35 is used in all calculations. Carbon distribution analysis along the sequence was obtained using CARBANA program available online (www.rajasekaran.net.in/tools/carbana.htm). A window size of 500 atoms was chosen in all calculations. The outputs obtained from CARBANA program were then plotted for better visualization of results. The active sites in the 3D structure of both wild-type and mutant proteins were analysed for average carbon density around the specified residues using CARd3D program. A radius of 10 Å units was used in the calculation. The results are tabulated and discussed.

RESULTS AND DISCUSSION

The structure 1J3I and 1J3K for the wild-type and mutated protein each has in total 4 chains. These are represented by 2 sequence-unique entities. The mutations are restricted in chain A which has length of 280 residues. From PMD database mutations sites identified were at amino acid residues 16, 51, 59, 108, and 164 corresponding to mutants namely A16V, N51I, C59R, S108N and I164L.

Carbon distribution at mutational sites of the protein

Analysis of the carbon distribution of the wild-type and mutant A16V is shown in Fig 1. Generally it is expected that the distribution curve be a normal and centered at 0.3145. Higher or lower of this value indicate that the stretch is in hydrophobic or hydrophilic region. Disturbance in the normal distribution is considered as unstable region. The A16V mutant showed an elevated carbon content to 0.314 from 0.257 of the wild-type. The high carbon content in this portion is due to changes in increasing size and hydrophobicity of the side chain from Ala to Val at amino acid residue number 16. The carbon content distribution of the S108N mutant (Fig. 2) also exhibited similar values for carbon content distribution of 0.314 that is high compared to 0.3 of the wild-type. Changes of amino acid at residue position 108 for S108N is towards increasing carbon fraction of the side chain and distribution peak height, shape and size of mutants being almost same. The addition of N at site 108 stabilises the local structure. The carbon content symmetry of the A16V and S108N mutants is shifting towards a normal distribution of the carbon content which is known to be symmetrical about 0.3145.

Carbon content for the N51I mutant (Fig. 3) showed the same peak value 0.257 with the wild-type interestingly, this shifting due to mutation had higher frequency compared to the wild-type and this is a result of substitution of N with I at amino acid position 51. This is an active site. Reduction of carbon content will reduce the activity. The carbon content value 0.314 observed for A16V and S108N mutants conform to stability of the protein at respective sites and are in agreement with established findings that stable and ordered proteins have a normal distribution of its carbon content symmetrical about 0.3145 (Rajasekaran and Vijayasarathy, 2011; Akila *et al.,* 2012).

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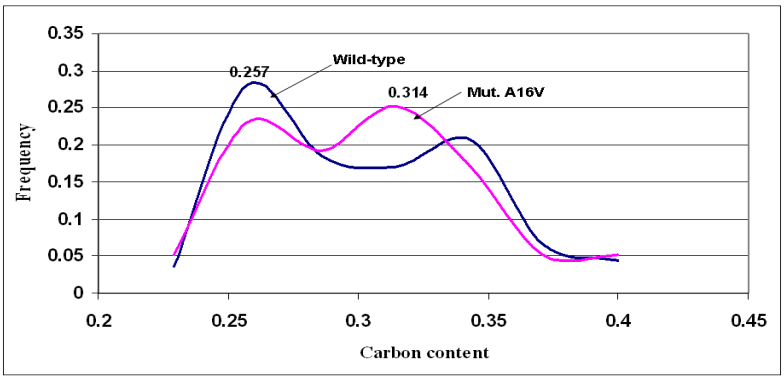

Fig 1: Carbon distribution for the Wild-type and mutant A16V of PfDHFR-TS protein

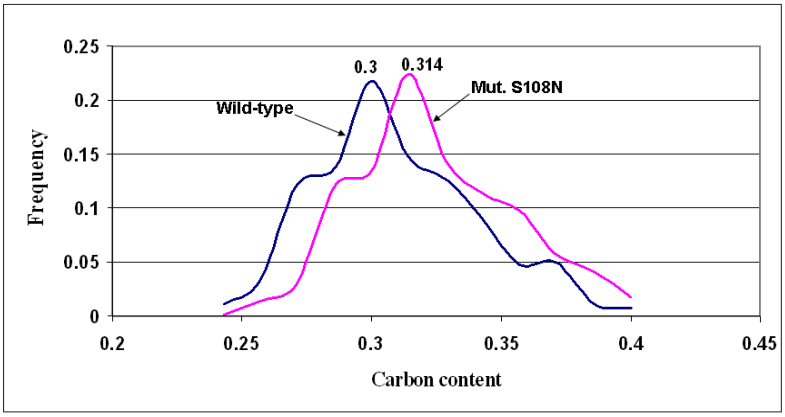

Fig 2: Carbon distribution for the Wild-type and mutants S108N of PfDHFR-TS protein

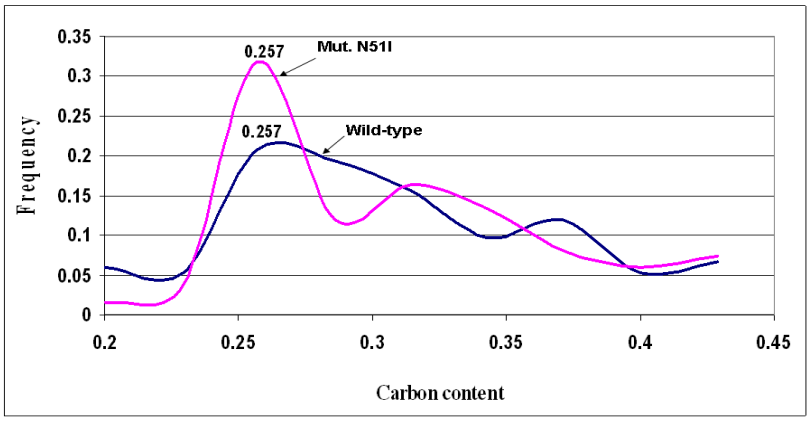

Fig 3: Carbon distribution for the Wild-type and mutant N51I of PfDHFR-TS protein

Carbon content distribution for mutant C159R (Fig. 4) showed high carbon content 0.371 compared 0.343 of the wild –type. Although this segment shows shifting of carbon content from low to high, but both peak positions for the wild-type and mutant are far from the symmetrical at 0.3145 for normal distribution of the carbon content. A peculiar result was obtained for mutants I164L (Fig. 5) which revealed similar peak at 0.343 with the wild-type for carbon content distribution. The similar pattern of the graph and peak could be attributed with changes of amino acid residues Ile to Leu at position 164 with the same value in relation to carbon content distribution. This is due to the side chains of I and L are same but isomers. This does not alter the carbon content or distribution at local structure. This mutation may not be required in any way.

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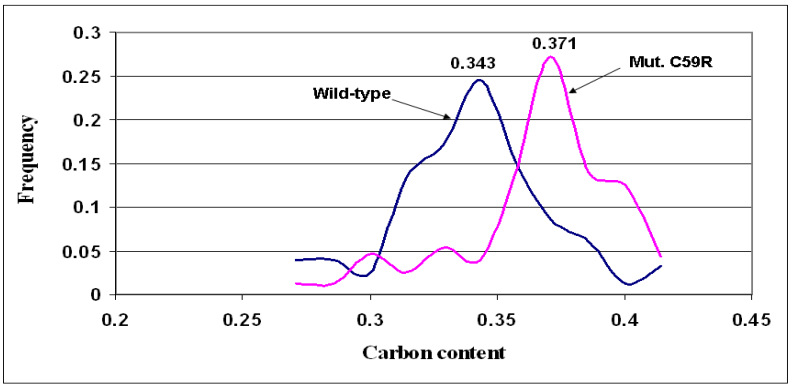

Fig 4: Carbon distribution for the Wild-type and mutant C159R of PfDHFR-TS protein

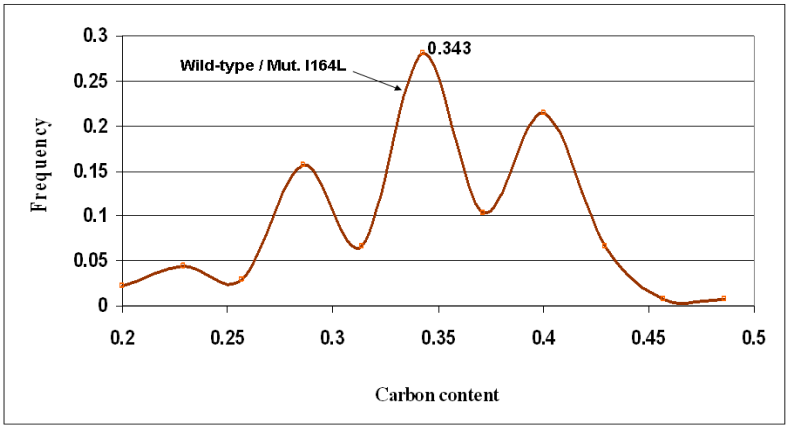

Fig 5: Carbon distribution for the Wild-type and mutants I164L of PfDHFR-TS protein

The results revealed that all portions of mutated PfDHFR-TS protein at A16V, N51I, C59R, S108N, S108T, and I164L had proportion of carbon content at the normal distribution about 0.3145 or more than 0.3145. It has previously been reported that a portion of protein sequence which has more than 0.3145 carbon content is considered as active sites (Senthil and Rajasekaran**,** 2009). For the wild-type protein (1J3I), pdbsum shows that the amino acid residues Ala 16, Ser 108 and Ile 164 are in the active sites. The pdbsum further reports that the same amino acid residue positions for the mutated protein (1J3K) are still in active sites of the protein indicating that mutation is tolerated at active sites. The presence of these active sites despite mutations is probably due to presence of high carbon content which will not affect enzyme activity. Thus, this has contributed to the efficacy of this class of DHFR-inhibitor drugs being compromised because of mutations. It has also previous been reported that the drug resistance mutation had no negative effect on the function of the enzyme (Yuvaniyama *et al.,* 2003; Lozovsky *et al.,* 2009). From structural analysis, it is observed that residue 59 involved in the interface of protein-protein interaction. Residues 108 and 164 are exposed and may be available for drug interaction.

Carbon distribution along the protein sequence and hydrophobicity

The carbon distribution profile obtained from CARBANA tool for the wild-type (1J3I) and mutant (1J3K) proteins is given in Fig. 6. The figure shows the percentage of carbon along the sequence.

Both plots for wild-type and mutant protein show a normal distribution of carbon along the protein sequence. The results still indicates that despite the mutations in 1J3K, its plot for mean distribution of carbon content is maintained at 31.45% of carbon all along the sequence.

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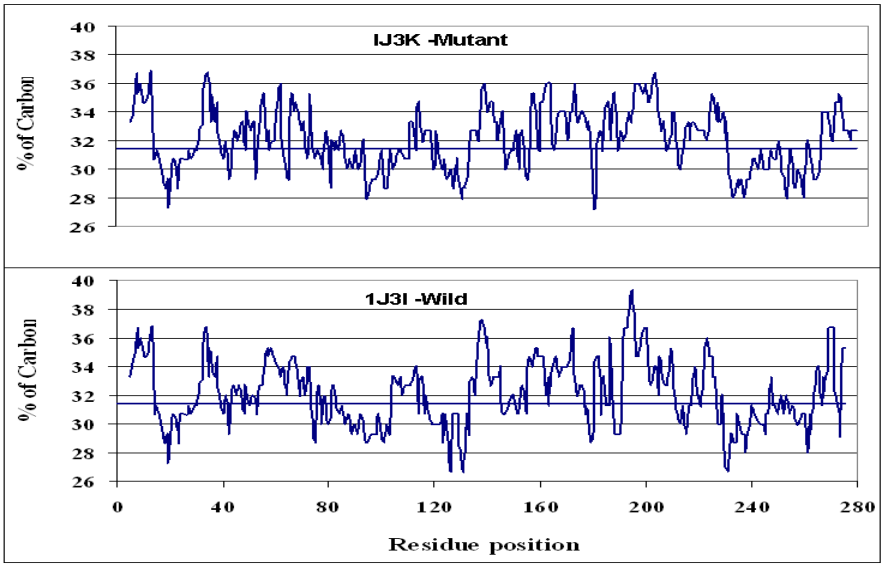

Fig 6: Carbon distribution profile along the sequence of wild-type and mutant proteins

Some regions along the sequences have values above 31.45%, these are considered to be higher carbon content or hydrophobic regions as it has previously been reported (Rajasekaran and Vijayasarathy, 2011). Thus, the overall hydrophobicity of all the proteins wild-type and mutant (1J3I and 1J3K) balance quite well for the entire sequence at the optimum position of stable proteins and carbon is the only element contributes towards this stability.

The mutational site A16 is not an active site as reported in PMD since it belongs to hydrophilic region (see fig 6). The mutation A16V adds carbon and stabilizes the local structure. All other reported sites (51, 59, 108 and 164) are in hydrophobic region which can be active site. Site 51 seems to be in neither hydrophobic nor hydrophilic region. It is not important for mutational study. As discussed earlier, the site 59 is in carbon rich region and in the interface of protein-protein association. Addison of R in place of C will certainly weaken the protein-protein interaction. The site 108 in wild-type form is in hydrophobic region while in mutant (S108N) form, it is in hydrophilic region. The binding of probe will not be effective in mutant protein compared to wild-type form. The mutation I164L does not alter carbon content because both amino acids have the same atomic content in the side chains. As noted earlier, there are presence of large hydrophobic residues at active sites of mutated protein such as A16V and I164L, these also might have contributed to stability of protein as previous been reported that proteins accumulate more large hydrophobic residues in active sites (Jayaraj *et al.,* 2009). Furthermore, Gromiha (2003) reported that the mutant stability is attributed with several factors including hydrophobicity.

Table 1: Average carbon fraction at the mutational sites with calculation radius of 10 Å.

Wild-tye (1J3I)	Mutant (1J3K)
0.657(N51)	0.659(N51I)
0.690(C59)	0.652(C59R)
0.658(S108)	0.671(S108N)
0.686(I108)	0.683(I164L)

The carbon distribution at the mutational sites in crystal structures of wild-type and mutant form is computed for radius of 10 Å. The average values are given in table 1. The mutation N51I does not alter the carbon content significantly. It is another proof of non active site. A remarkable change in carbon distribution around the site 59 is observed due to mutation. That reduction in carbon fraction is observed. The mutation is significant but reduction in activity. A moderate increase in hydrophobicity in site 108 is observed due to mutation. This mutation will improve the binding capability. Merely any change in carbon distribution at site 164 due to mutation. The mutation is not significant and does not alter the activities. Carbon distribution study can identify mutational sites and suggest amino acids for mutation for stable and active protein.

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CONCLUSION

This study has revealed that despite mutations, the carbon content and distribution at mutational sites and along the protein sequences of *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) is still stable, consequently retaining its enzymatic activity. Thus, the carbon distribution study along the protein chain is the most significant step towards understanding the biological features which can provide possible approaches for the design of new drugs to overcome antifolate resistance. Carbon distribution study can identify mutational sites and suggest amino acids for mutation for stable and active protein.

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REFERENCES

Akila, K., Sneha N. and Rajasekaran E. (2012). Study on carbon distribution at protein regions of disorder, Intern. J. Biosc., Biochem. and Bioinf., 2(2): 58–60.

Chusacultanachai, S., Thiensathit P., Tarnchompoo B., Sirawaraporn W. and Yuthavong Y. (2002). Novel antifolate resistant mutations of *Plasmodium falciparum* dihydrofolate reductase selected in *Escherichia coli*. Mol Biochem Parasitol. 120(1):61–72.

Dasgupta, T., Chitnumsub P., Kamchonwongpaisan S., Maneeruttanarungroj C., Nichols S.E., Lyons T.M., Tirado-Rives J., Jorgensen W.L., Yuthavong Y. and Anderson K.S. (2009). Exploiting structural analysis, in silico screening, and serendipity to identify novel inhibitors of drug-resistant falciparum malaria ACS Chem. Biol. 4:29-40

Gromiha, M.M., Uedaira H, An J., Selvaraj S., Prabakaran P. and Sarai A. (2002). ProTherm, thermodynamic database for proteins and mutants: developments in version 3.0. Nucleic Acids Res. 30:301–2.

Gromiha, M.M. (2003). Factors influencing the thermal stability of buried protein mutants. Polymer 44: 4061–4066

Jayaraj, V., Suhanya R., Vijayasarathy M., Anandagopu M. and Rajasekaran, E. (2009). Role of large hydrophobic residues in proteins Bioinformation 3(9): 409-412

Kwiatkowski, D.P (2005). How malaria has affected the human genome and what human genetics can teach us about malaria. Am. J. Hum. Genet. 77 (2): 171–92.

Lozovsky, E.R., Chookajorn T., Brown K.M., Imwong M., Shaw P.J., Kamchonwongpaisan S., Neafsey D.E., Weinreich D.M.and Hartl D.L. (2009). Stepwise acquisition of pyrimethamine resistance in the malaria parasite. Proc. Natl. Acad. Sci. 106:12025-12030.

Murray, J.L.C., Rosenfeld C.L., Lim S.S., Andrews K.G., Foreman K.J., Haring D., Fullman N., Naghavi M., Lozano R. and Lopez D.A. (2012). Global malaria mortality between 1980 and 2010: a systematic analysis. The Lancet, 379: (9814): 413 – 431.

Rajasekaran, E. and Vijayasarathy M. (2011). CARBANA: Carbon analysis program for protein sequences. Bioinformation 5(10): 458–457.

Senthil, R. and Rajasekaran E. (2009) Comparative analysis of carbon distribution and hydropathy plot. J. Adv. Biotech., 8: 30-31

Tipsuwan, W., Srichairatanakool S., Kamchonwongpaisan S., Yuthavong Y. and Uthaipibull C. (2011). Selection of drug resistant mutants from random library of *Plasmodium falciparum* dihydrofolate reductase in *Plasmodium berghei* model. Malaria Journal 10:119

WHO, 2011. World malaria report 2011. WHO Press, Geneva Switzerland.

WHO, 2008. World malaria report 2008. WHO Press, Geneva Switzerland.

Yuthavong, Y. (2002). Basis for antifolate action and resistance in malaria. Microbes Infect 4:175-182.

Yuvaniyama, J., Chitnumsub P., Kamchonwongpaisan S., Vanichtanankul J., Sirawaraporn W., Taylor P., Walkinshaw M.D. and Yuthavong Y. (2003). Insights into antifolate resistance from malarial DHFR-TS structures. Nat. Struct. Biol. 10:357-365