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Structural Studies on Mitogen Activated Protein Kinase from Plasmodium Falciparum

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Abstract

Human malaria is caused by the apicomplexan, Plasmodium falciparum. Malaria causes the major health problems in most of the developing countries, including India. In addition, increased resistant against existing drugs of malaria, impose the identification of novel drug targets. Plasmodium kinome has been studied in detail so far, providing key insight into the major differences between human and malaria parasite kinases. Few kinases are essential in asexual life stage of the parasite and do not club together with human counterparts in phylogenetic tree. Mitogen-activated protein kinase-2 (Pfmap-2) is one of the two MAP kinases found in Plasmodium and essential for the asexual growth. In this study, we have structurally characterized the Pfmap-2 using in-silico homology modeling. Also the structural comparison was performed between parasite and human counterparts. In addition, active site of Pfmap-2 was identified using online tool. Together, we think that present study will definitely hasten the process of development of new and effective anti-malaria drugs.

Keywords: Kinase, MAPK, molecular modelling, malaria, drug discovery.

Introduction

Malaria is one of the major problems in many developing countries, including India, caused by the apicomplexan parasite Plasmodium. Several endemics of the malaria have been reported. There is no viable vaccine for malaria and available drugs are not effective enough due to increased resistant in malaria parasite. The present situation raised the concern of identifying new protein molecules which can be treated as potential drug target. There are many pathways important for parasite survival and some of them are very unique to parasite that needs to be explored. Protein kinases play a crucial role in development and regulation of cell growth[1]. Plasmodium kinome has been identified. The number of genes coding for kinases are smaller in size when compared to human counterparts[2-3]. There are several unique and different kinases present in malaria parasite which are absent in human host, makes them suitable targets for drug development[4]. Mitogen-activated protein kinases (MAPKs) family play major role in differentiation of eukaryotic cell. Plasmodium harbours only two members of this family, Pfmap-1 and Pfmap-2[6]. Both the kinases are important in schizogony, gametocytogenesis and gametogensis[7-8]. Pfmap-1 functions can be complemented by Pfmap-2 protein but not vice-versa. Hence, Protein Pfmap-2 is highly essential for the development of asexual life cycle of the parasite[9-10]. Moreover, Pfmap-2 branches off the cluster of eukaryotic MAPKs members in a phylogenetic study conducted, makes it suitable target for chemotherapy[11]. Therefore, in present work, we have solved the three-dimensional structure of Pfmap-2 via molecular modeling and also deciphered the probable active site of the kinase. In addition, comparison of modelled structure of Pfmap-2 with its human counterpart provided many spatial differences which could be utilized for better chemotherapy development against malaria parasite.

Material and Methods

The sequence of Pfmap-2 was extracted from PLASMODB using PF11_0147 as accession number. 1BMK pdb structure was used as a template for homology modeling. Identification of template structures was carried out using NCBI BlastP where search parameters were restricted to PDB (Protein Data Bank). Sali's Modeller and Swiss Model Server were used to build the in-silico structure of Pfmap-2. Online facility of sequence submission and locally downloaded program of Modeller, both were used to construct three-dimensional structure of Pfmap-2. RAMPAGE[12] online server was used for structure validation which gives output of Ramachandran plot describing maximum allowed amino acids present in modelled structure. Structural comparison was performed using CHIMERA[13]. Active site prediction was performed with CASTp using modelled structure of Pfmap-2 protein[14]. Images were created using CHIMERA. Images were processed at higher resolution in PNG format.

Results and Discussion

The structure of Pfmap-2 contains typical MAPK domain where half of the structure is made up of beta strands whereas remaining half is dominated with the presence of alpha helices (figure-1). In addition there are several loops hanging out of the core part of structure probably involved in making protein-protein interaction, needed for phosphorylation process. Panel B of fig.1 shows the surface topology of Pfmap-2 where surface is evenly distributed between negatively and positively charged

residues. However, positively charged residues may be involved in accommodating negatively charged ATP molecules, a source of phosphate moiety for kinase reaction. Ramachandran plot of the modelled Pfmap-2 suggest that most of the amino acid residues are in allowed region of three-dimensional space and thus validate the homology modeling (figure-2). Further, structural comparison between Pfmap-2 of human and P. falciparum was performed. Overall the both the proteins share common fold and domain topology but there are few structural differences like one the helix of Pfmap-2 is positioned close to the active site cavity when compared with human kinase, subtle changes in three-dimensional space of helices and loops (figure-3). An extended loop was only present in Pfmap-2 not in human counterpart. This insertion of sequence motifs in plasmodium proteins is common phenomenon results in increase in the length of protein, although, functional significance is not yet known. These structural differences could become basis of drug development strategy as small differences in three-dimensional space are enough for an inhibitor to bind with variable affinity. Computed Atlas of Surface Topography of Proteins (CASTp) provided the predicted active site location within Pfmap-2. The amino acid residues which make the active site pocket are coloured in green and there 3D-space locations are highlighted both in ribbon and surface diagram (figure-4). The active site volume and area are 1792.5 Ao and, 2403.9, very large in comparison to other enzymes, good enough to hold any protein along with ATP molecules to carry out phosphorylation.

Conclusion

Kinase proteins are very important for cell's development and differentiations. Plasmodium kinome revealed two major MAPKs involved in the development of asexual life cycle of parasite. Biochemical characterization of these two kinases was already done but to understand the phosphorylation process better and to utilize the structural discrepancy between human host and parasite, three-dimension structure of Pfmap-2 was necessary. To do so, we adopted relatively easy and rapid method of solving three-dimensional structure using molecular modeling. Homology modeling of Pfmap-2 provided the much needed structural information required to understand involvement of this protein in known biological processes. For instance, structure revealed the large cavity of the proteins as a probable active site, large enough to accommodate target protein and ATP molecule. Also the positively charged residues near active site could be required for binding to highly negative ATP molecules. In addition, there were many structural disparity between Pfmap-2and human counterparts, may be taken as initiative for in-silico drug screening, probably lead to the identification of novel anti-malarial specific drug.

Acknowledgement

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References

1. Cohen P., The role of protein phosphorylation in human health and disease, *Eur J Biochem*, **268**, 5001–5010 **(2001)**

2. Anamika X., Srinivasan N. and Krupa A., A genomic perspective of protein kinases in Plasmodium falciparum, *Proteins*, **58**, 180–189 **(2005)**

3. Ward P., Equinet L., Packer J. and Doerig C., Protein kinases of the human malaria parasite Plasmodium falciparum: the kinome of a divergent eukaryote, *BMC Genomics*, **5**, 79 **(2004)**

4. Doerig C. and Meijer L., Antimalarial drug discovery: targeting protein kinases, *Expert Opin Ther Targets*, **11**, 279–290 **(2007)**

5. Doerig C.M., Parzy D., Langsley G., Horrocks P., Carter R. and Doerig C.D., A MAP kinase homologue from the human malaria parasite, Plasmodium falciparum, *Gene*, **177,** 1–6 **(1996)**

6. Graeser R., Kury P., Franklin R.M. and Kappes B., Characterization of a mitogen-activated protein (MAP) kinase from Plasmodium falciparum, *Mol Microbiol*, **23,** 151–159 **(1997)**

7. Graves P.M., Carter R. and McNeill K.M., Gametocyte production in cloned lines of Plasmodium falciparum, *Am J Trop Med Hyg*, **33,** 1045–1050 **(1984)**

8. Dorin D., et al., An atypical mitogen-activated protein kinase (MAPK) homologue expressed in gametocytes of the human malaria parasite Plasmodium falciparum. Identification of a MAPK signature, *J Biol Chem*, **274**, 29912–29920 **(1997)**

9. Rangarajan R., Bei A.K., Jethwaney D., Maldonado P., Dorin D., Sultan A.A. and Doerig C., A mitogenactivated protein kinase regulates male gametogensis and transmission of the malaria parasite Plasmodium berghei, *EMBO Rep*, **6,** 464–469 **(2005)**

10. Tewari R., Dorin D., Moon R., Doerig C. and Billker O., An atypical mitogen-activated protein kinase controls cytokinesis and flagellar motility during male gamete formation in a malaria parasite, *Mol Microbiol*, **58**, 1253–1263 **(2005)**

11. Ward P., Equinet L., Packer J. and Doerig C., Protein kinases of the human malaria parasite Plasmodium falciparum: the kinome of a divergent eukaryote, *BMC Genomics*, **5**, 79 **(2004)**

12. Lovell S.C., Davis I.W., Arendall W.B. , de Bakker P.I.,

Word J.M., Prisant M.G., Richardson J.S. and Richardson D.C., Structure validation by Calpha geometry: phi,psi and Cbeta deviation, *Proteins.*, **15, 50(3)**, 437-50 **(2000)**

13. Dundas J., et. Al., CASTp: computed atlas of surface topography of proteins with structural and topographical mapping of functionally annotated residues, *Nucleic Acids Res.*, **34,**116–118 **(2006)**

14. Pettersen E.F., Goddard T.D., Huang C.C., Couch G.S., Greenblatt D.M., Meng E.C. and Ferrin TE, UCSF Chimera - A Visualization System for Exploratory Research and Analysis, *J Comput Chem.,* **25**,1605-1612 **(2004)**

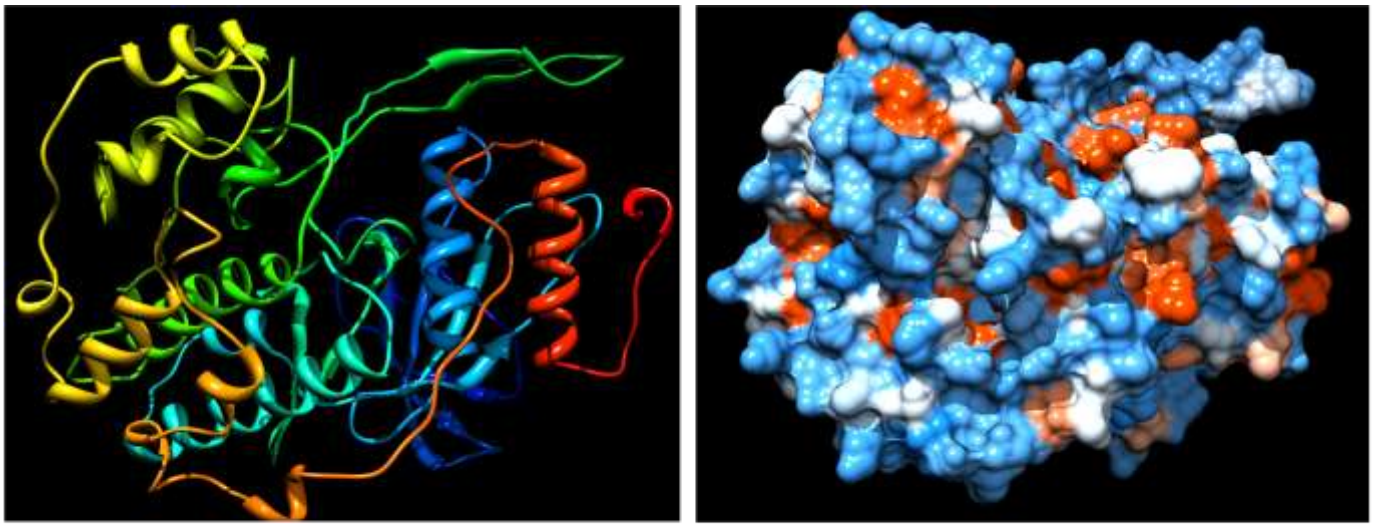

Figure 1: Three-dimensional structure of Pfmap-2. Left panel showing modelled structure in ribbon form whereas right panel display hydrophobic surface.

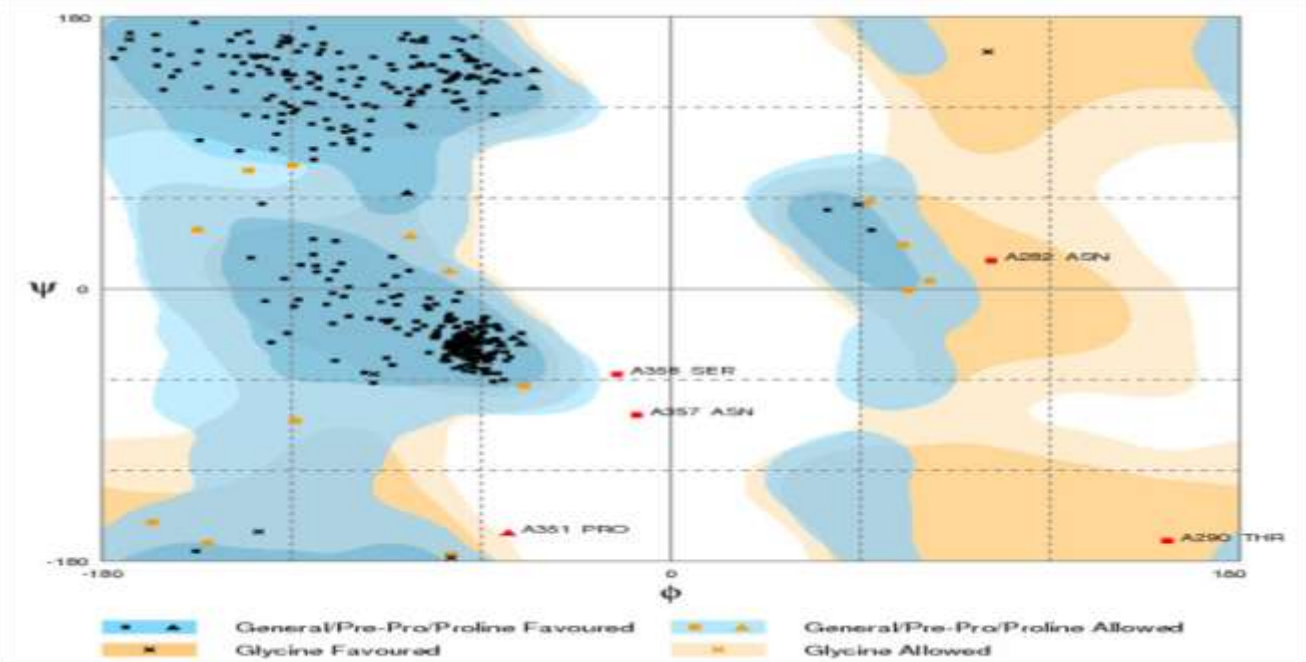

Figure 2: Ramachandran plot of modelled Pfmap-2 structure

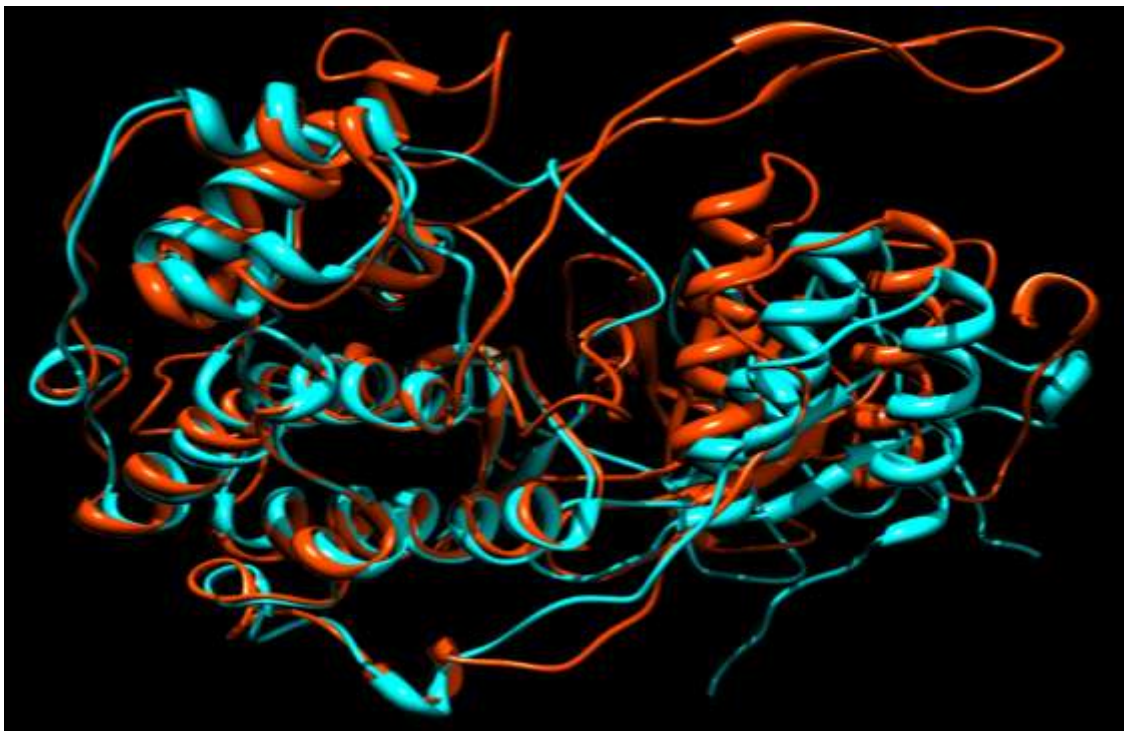

Figure - 3
Structural comparison between human and Plasmodium protein Pfmap-2

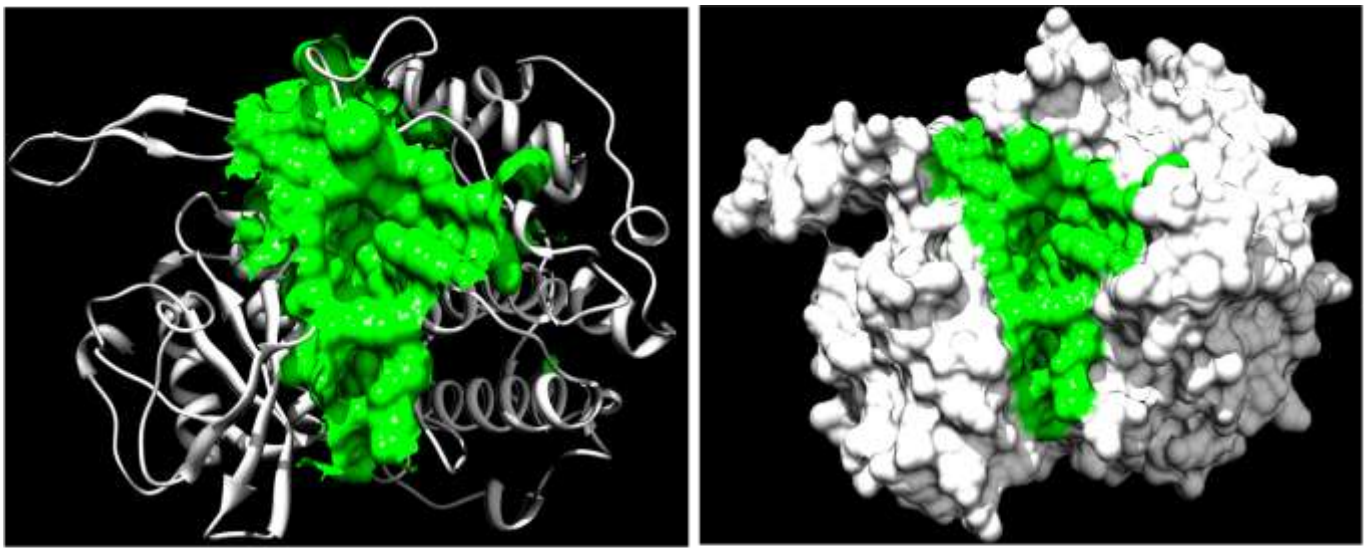

Figure 4: Predicted active site of Pfmap-2. Upper panel showing the protein sequence of modelled P43 structure where active site residues are labelled in green. Lower panel shows the active site pocket of Pfmap-2 in 3D space with ribbon and surface diagram.

Chain A																																																		
95-	A	I	I	K	N	V	K	V	P	D	N	Y	E	I	K	H	L	I	G	R	G	S	Y	G	Y	V	Y	L	A	Y	D	K	N	A	N	K	N	V	A	I	K	K	V	N	R	M	F	E	D	L
145-	I	D	C	K	R	I	L	R	E	I	T	I	L	N	R	L	K	S	D	Y	I	I	R	L	H	D	L	I	I	P	E	D	L	L	K	F	D	E	L	Y	I	V	L	E	I	A	D	S	D	L
195-	K	K	L	F	K	T	P	I	F	L	T	E	Q	H	V	K	T	I	L	Y	N	L	L	L	G	E	K	F	I	H	E	S	G	I	I	H	R	D	L	K	P	A	N	C	L	L	N	Q	D	C
245-	S	V	K	I	C	D	F	G	L	A	R	T	I	N	S	D	K	D	I	H	I	V	N	D	L	E	E	K	E	E	N	E	E	P	G	P	H	N	K	N	L	K	K	Q	L	T	S	H	V	V
295-	T	R	W	Y	R	A	P	E	L	I	L	L	Q	E	N	Y	T	N	S	I	D	I	W	S	T	G	C	I	F	A	E	L	L	N	M	M	K	S	H	I	N	N	P	T	N	R	F	P	L	F
345-	P	G	S	S	C	F	P	L	S	P	D	H	N	S	K	K	V	H	E	K	S	N	R	D	Q	L	N	I	I	F	N	V	I	G	T	P	P	E	E	D	L	K	C	I	T	K	Q	E	V	I
395-	K	Y	I	K	L	F	P	T	R	D	G	I	D	L	S	K	K	Y	S	S	I	S	K	E	G	I	D	L	L	E	S	M	L	R	F	N	A	Q	K	R	I	T	I	D	K	A	L	S	H	P
445-	Y	L	K	D	V	R	K	E	N	L	E	N	F	S	T	E	K	I	I	L	P	F	D	D	W	M	V	L	S	E	T	Q	L	R	Y	I	F	L	K	E	I	Q	S	F	H	A	D	L	I	I
495-	P	A	K	L	N																																													