ijcrr
Vol 04 issue 08
Category: Research
Received on:20/03/12
Revised on:23/03/12
Accepted on:27/03/12

IN-SILICO STUDIES ON P43 PROTEIN FROM PLASMODIUM FALCIPARUM

Tarun Kumar Bhatt

Department of Biotechnology, Central University of Rajasthan, Kishangarh

E-mail of Corresponding Author: tarunbhatt1982@gmail.com

ABSTRACT

Eukaryotic Aminoacyl-tRNA synthetases exist in large complex consists of different tRNA synthetases with auxiliary proteins. P43 is one of the three non-synthetases proteins found in multi-synthetases complex. P43 has been shown to involve in various biological processes like tRNA transport from nucleus, apoptosis etc. Homologous sequence of P43 is also found in *Plasmodium falciparum* (PfP43). In this study, homology modeling, structure validation and active site determination methods were used to perform structural characterization of P43. Results show the overall three-dimensional structure of P43 with proper Ramachandran plot. Also, Active site residues were nicely located onto the structure of P43. In addition, structural comparison between P43 of human and parasite origin provided information on subtle differences in overall structures of proteins. Our results suggest that elucidation of PfP43 structure is critical in developing anti-malarial drugs.

Keywords: P43, Homology modeling, Plasmodium

INTRODUCTION

Plasmodium falciparum is the causative agent of epidemic disease malaria. Many developing countries are suffering from socio-economic burden of this fatal parasitic disease. Several drugs have been identified against malaria parasite but prime concern remains are the rapid development of drug resistance among parasites. In addition, *P. falciparum* adapts different strategies to overcome immune responses[1-4] and because of it, effective vaccine against parasite has not been developed. Taking into consideration all the facts discussed above, there is regular need of identifying new protein molecules of the parasite which could be targeted as potential drug target.

Aminoacyl-tRNA synthetases (aaRSs) are conserved class of proteins which play important role in protein synthesis machinery of all living organism. In eukaryotes, aaRSs are found in the form of multi-synthetases complex (MSC), comprises of 9-10 different tRNA synthetases and 3 non-synthetases proteins[5]. P43 is part of the non-synthetases component of MSC. Protein P43 has been shown to involve in different biological processes which include trafficking of tRNA, involvement in autoimmune disease, inhibition of formation of new vascular tissue in metastatic carcinoma and in stability to MSC[6-9]. In addition, the important function of P43 comes into play as precursor of EMAPII (Endothelial Monocyte Activating Polypeptide) domain.

EMAPII is involved in acute inflammation and play crucial role in apoptotic processes[10]. This aaRSs family of proteins have been identified in Plasmodium along with the homolog of P43 (PfP43). Nothing much has been done in characterization of this protein but PfP43 was found to be secretory in nature during parasite asexual life cycle in human. Pro-inflammatory property of PfP43 might play an important role in modulating host immune response and could be vital in malaria patho-physiology. In this work, we have utilized the quick and effective method of solving three-dimensional structure of PfP43 using homology modeling. Comparative studies with human counterparts along with the identification of active site of the protein P43 could pave the way in identifying new effective drug-like molecules against deadly malaria parasite.

MATERIALS AND METHODS

The sequence of PfP43 was obtained using NCBI Blast by taking human counterpart as template. Other information of PfP43 was extracted from PLASMODB using PF14_04013 as accession number. 1E7Z and 1FL0 pdb structures were 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 PfP43. Online facility of sequence submission and locally downloaded program of Modeller, both were used to construct three-dimensional structure of P43 domain. RAMPAGE online server was used for structure validation which gives output of Ramachandran plot describing maximum allowed amino acids present in modelled structure. Active site prediction was performed with CASTp using modelled structure of PfP43 domain. Images were created using CHIMERA[11]. Images were processed at higher resolution in PNG format.

RESULTS AND DISCUSSION

The three-dimensional structure of PfP43 domain is highly compact in nature and it is typically EMAPII like domain. Structure is the mixture of alpha helices and beta sheets where beta sheets are predominantly occupying the most of the space (fig.1). In addition there are several loops hanging out of the core part of structure probably involved in making contact with interacting molecules which include both protein and nucleic acid in case of P43. Panel B of fig.1 shows the surface topology of PfP43 where most of the surface is positively charges with intermittent negative charged patches, indicative of nucleic acid binding ability of PfP43. However, one side of the PfP43domain is highly negative in nature typically nucleic acid binding site whereas other side is mixture of both negative and positively charged residues which might be interacting with other proteins based on charge complementarily. Ramachandran plot of the modelled PfP43 suggest that most of the amino acid residues are in allowed region of three-dimensional space and thus validate the homology modeling (fig.3). Further, structural comparison between P43 of human and Plasmodium was performed. Overall the both the proteins share common fold and domain topology but there are few structural differences like secondary structure of beta sheet present in PfP43 whereas absent in human counterpart, subtle changes in three-dimensional space of helices and loops (fig.2). 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 PfP43. 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 (fig.4). The active site volume and area are 149 A^o and 176.9, good enough to accommodate one or two bases in case on nucleic acid and two or three amino acids in case of proteins.

CONCLUSION

To understand the mechanism of enzyme reaction or binding of two protein molecules, structural information play a Very critical role, and to get the structure of proteins using X-ray crystallography or NMR or Electron microscopy is very expensive and time consuming process. Thereby, we adopted relatively cheap and fast method of solving three-dimensional structure using molecular modeling. Homology modeling of PfP43 provided the much needed structural information required to understand involvement of this protein in many biological processes. For example, occurrence of highly negative patches on one side of protein led us to speculate the tRNA binding region which is necessary for the function of transport of tRNA molecules out of the nucleus as well as for the stable formation of multi-synthetases complex. Note only that, remaining area of protein in three-dimensional space with variable charge distribution might be responsible for binding to other cellular factors engaged in apoptosis or inflammatory pathways. In the end, structural differences between PfP43and human counterparts might pave the way for in-silico screening which might lead to malaria specific drug like molecules discovery.

ACKNOWLEDGEMENT

I would like to thank Central University of Rajasthan, Department of Biotechnology for providing resources to conduct these studies. I also thank Deepti Joshi for her help in performing homology modeling. Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

REFERENCES

1. Smith JD, Chitnis, CE, Craig AG, Roberts DJ, Hudson-Taylor DE, Peterson S, Pinches R, Newbold CI and Miller LH. Switches in expression of *Plasmodium* falciparum var genes correlate with changes in antigenic and cytoadherent phenotypes of infected erythrocytes. Cell, 1995, 82:101–110.
2. Florens L, Liu X, Wang Y, Yang S, Schwartz O, Peglar M, Carucci DJ, Yates JR, 3rd, and Wub Y. Proteomics approach reveals novel proteins on the surface of malaria-infected erythrocytes. Mol. Biochem. Parasito 2004, l135:1–11.
3. Gazzinelli RT, and Denkers EY. Protozoan encounters with Toll-like receptor signalling pathways: implications for host parasitism. Nat. Rev. Immunol. 2006, 6:895–906.
4. Jangpatarapongsa K, Chootong P, Sattabongkot J, Chotivanich K, Sirichaisinthop J, Tungpradabkul S, Hisaeda H, Troye-Blomberg M, Cui L, and Udomsangpetch R. *Plasmodium* vivax parasites alter the balance of myeloid and plasmacytoid dendritic cells and the induction of regulatory T cells. Eur. J. Immunol. 2008, 38:2697–2705.
5. Kerjan P, Cerini C, Semeriva M, Mirande M. The multienzyme complex containing nine aminoacyl-tRNA synthetases is ubiquitous from Drosophila to mammals. Biochem Biophys Acta 1994, 1199:293-297.
6. Han JM, et al. Aminoacyl-tRNA synthetase interacting multifunctional protein 1/p43 controls endoplasmic reticulum retention of

heat shock protein gp96: Its pathological implications in lupus-like autoimmune diseases. Am J Pathol 2007, 170:2042–2054.
7. Liu B, et al. Cell surface expression of an endoplasmic reticulum resident heat shock protein gp96 triggers MyD88-dependent systemic autoimmune diseases. Proc Natl Acad Sci USA 2003, 100:15824–15829.
8. Park SG, et al. Dose-dependent biphasic activity of tRNA synthetase-associating factor, p43, in angiogenesis. J Biol Chem 2002, 277:45243–45248.
9. Lee YS, et al. Antitumor activity of the novel human cytokine AIMP1 in an in vivo tumor model. Mol Cells 2006, 21:213–217.
10. Ko HS, et al. Accumulation of the authentic parkin substrate aminoacyl-tRNA synthetase cofactor, p38/JTV-1, leads to catecholaminergic cell death. J Neurosci 2005, 25:7968–7978.
11. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC and Ferrin TE. UCSF Chimera - A Visualization System for Exploratory Research and Analysis, J Comput Chem. 2004, 25:1605-1612.

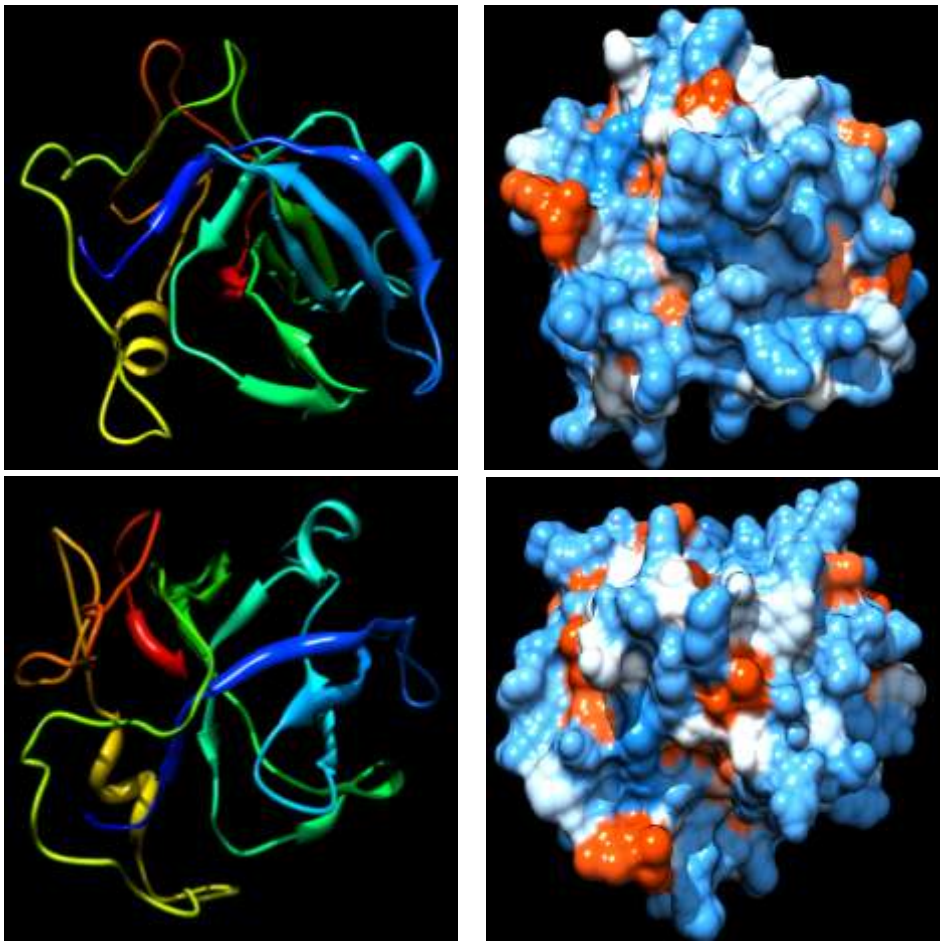

Figure 1: Three-dimensional structure of PfP43. Left panel showing modelled structure in ribbon form whereas right panel display hydrophobicity surface in different directions

Figure 2: Structural comparison between human and Plasmodium protein P43 domain AIMPII

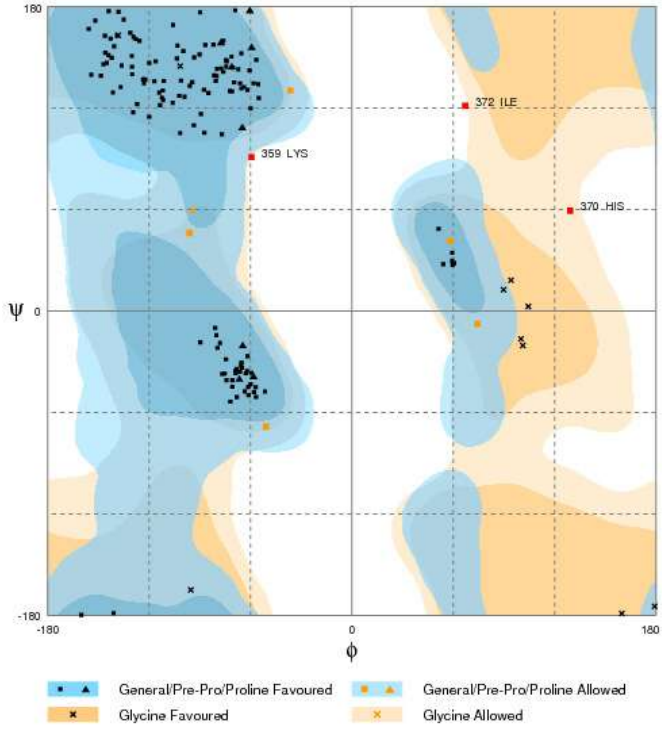

Figure 3: Ramachandran plot of modelled P43 structure

242- S R L N V L V G Y V E Q V E I H P D A D T L Y C L K I N L G E D K P R D I C S G L R N K K N A E D L
292- L N K Y V L V L A N L K E K S L R G K K S H G M V L C G S F D E K V E L L V P P N G V K I G E R I L
342- F H N M D P N V I P D K N L S S D K E K N P F F H I Q P H L I L K D G V A H Y K D T K W I S S Q G D
392- I T C V L N Q G T I S

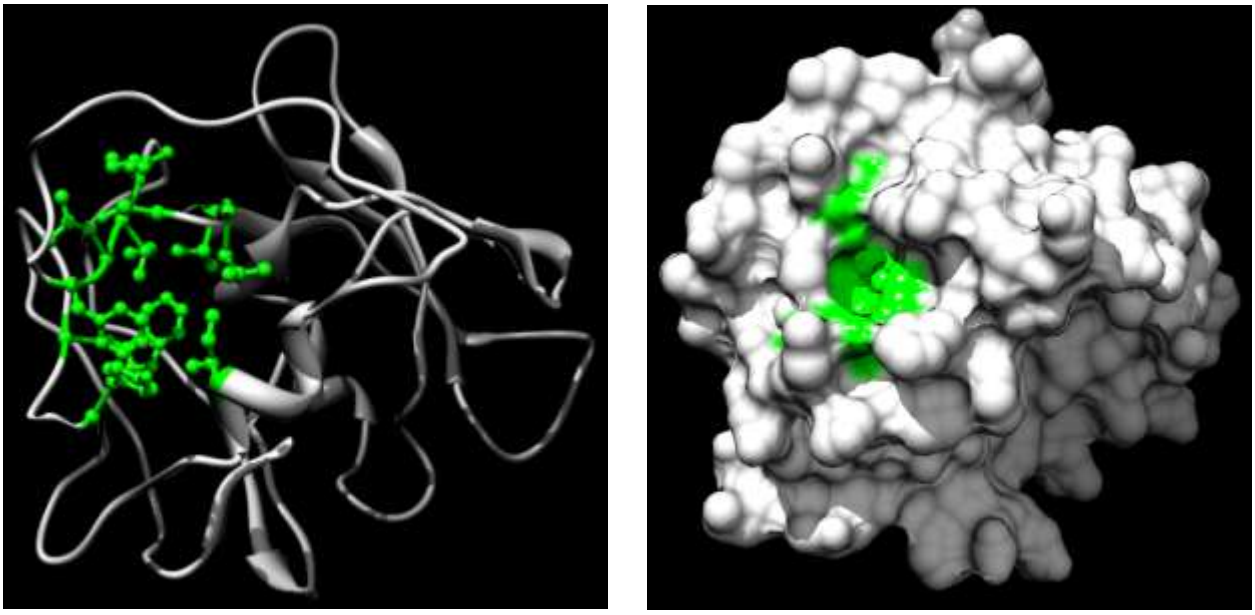

Figure 4: Predicted active site of P43. Upper panel showing the protein sequence of modelled P43 structure where active site residues are labelled in green. Lower panel shows the active site pocket of P43 in 3D space in ribbon and surface diagram.