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Short Communication

In-Silico Structure Determination of Protein Falstatin from Malaria Parasite *Plasmodium Falciparum*

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Abstract

Malaria is the major cause of socio-economic loss to most of the developing countries. Several drugs have been developed against the deadly malaria causing protozoan, Plasmodium falciparum. However, development of drug resistance against existing drugs has necessitated the identification of new drug targets. Several proteases have been identified from malaria parasite which is involved in various processes like haemoglobin degradation, egress of merozoite etc. But more important aspect of malaria biology is the regulation of these proteases for effective regulation of parasite life cycle. Falstatin is such a protein which binds to many cysteine proteases and regulates their activities. Therefore, Falstatin is the potential target for drug discovery. In this study, we determined the three-dimensional structure of Falstatin by molecular modelling using Swiss Modeller and Sali's Modeller. Ramachandran plot was used for structure validation. Falstatin active site was determined using CastP. Structural analysis of Plasmodium Falciparum Falstatin (Pf-Falstatin) could be instrumental in identifying new drug like molecules.

Keywords: Falstatin, cysteine proteases, molecular modelling, malaria, drug discovery.

Introduction

Malaria is one of the most devastating infectious diseases in the world. More than 100 million deaths are reported annually (WHO report, 2009). *Plasmodium* is the causative agent of the malaria disease. Several species of genus *Plasmodium* are present in the nature, out of which four species infect only human (*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale* and *Plasmodium malariae*). The life cycle of malaria parasites is complex, with the asexual stages occurring in mammalian hosts and the sexual stages in female Anopheles mosquito vectors. Malaria is transmitted by the bite of a mosquito in which hundreds of sporozoites are released into the host bloodstream. The parasites eventually migrate to the liver, passing through cell types such as Kupfer cells and form parasitophorous vacuoles in hepatocytes. At this stage, they can either remain dormant as a hypnozoite form (P. vivax or P. ovale), or initiate development that results in the production of thousands of merozoites. The parasites then released from the infected hepatocyte and invade erythrocytes where they replicate in a cycle that may correspond to the cycle of fever and chills in malaria. Some parasites differentiate into male and female gametocytes, which are the forms taken up by the mosquito where sexual life of parasite continues.

Genome sequencing of the *Plasmodium falciparum* has been almost completed. There are over 30 predicted sequences of cysteine proteases[1]. Several of them have been biochemically characterized[2]. These cysteine proteases are shown to involve in various critical processes like haemoglobin degradation, erythrocyte egression and erythrocyte invasion of merozoites[3-9]. However, regulation of these proteases is very important in terms of proper nutrition for the parasite inside host cell and also for the infection to be established. Pf Falstatin is one of the endogenous regulators of the cysteine proteases in the malaria parasite similar to the Chagasin, a cysteine proteases inhibitor in *Trypanosoma cruzi*[10-11]. PfFalstatin is known to inhibit many cysteine proteases and specifically inhibit PfFalcipain 2 proteases by binding to the active site of the falcipain[2] enzyme[12]. Temporal expression of this parasite protein is very critical in invasion of erythrocyte by merozoites[13-15]. Thus, elucidation of three-dimensional structure of PfFalstatin will not only help in the understanding of erythrocyte invasion but also lay the foundation of identifying new effective inhibitors against the malaria parasite.

Material and Methods

The sequence of PfFalstatin was retrieved from PlasmoDB (PFI0580c). 3PNR and 2C34 pdb structures were used as a template for homology modeling. Identification of template structures was carried out using NCBI BlastP. Sali's Modeller[16] and Swiss Model Server were used to build the in-silico structure of PfFalstatin. Structure validation was performed with ramachandran plot using online server rampage[17]. Modelled structure of PfFalstatin was submitted to CASTp for active site prediction[18]. Figures and images were developed using chimera[19].

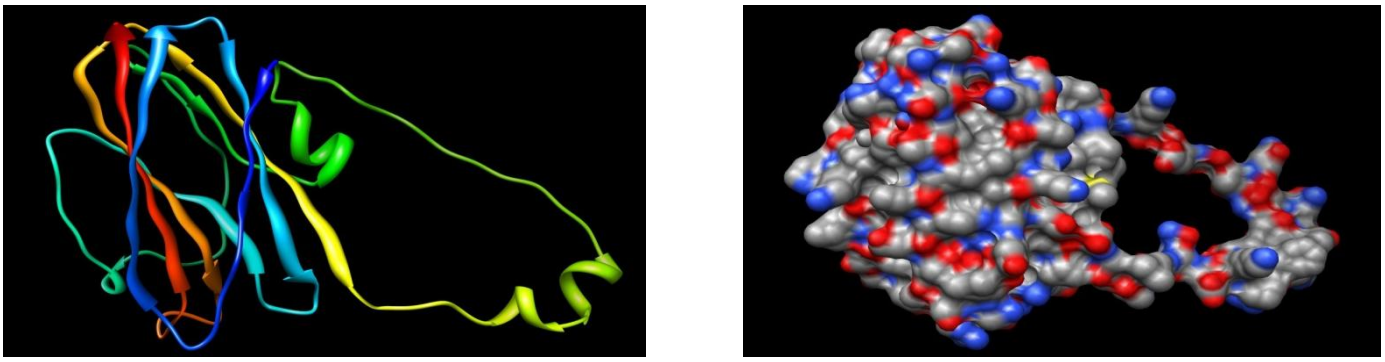
Results and Discussion

The modelled structure of PfFalstatin is shown in figure 1. Panel A represent the ribbon diagram of structure while panel B

shows the surface topology of PfFalstatin. Helices, sheets are present along with loops in the structure. An extended loop is also present with short helix in it. Surface topology diagram shows that most of the surface is positively charges with intermittent negative charged patches. When structure of PfFalstatin was compared with its homologous structure of *Plasmodium berghei*, several distinct features were observed in spite of similar fold of the proteins (figure 2). Two helices were missing in the P. berghei falstatin structures compared to PfFalstatin, where both the helices were nicely build and modelled. In addition, structure validation was done by using ramachandran plot which shows that most of the residues are in favoured and allowed region (figure 3). Further, prediction of active site of PfFalstatin was carried out with CASTp (figure 4). Computed atlas of surface topography of proteins (CASTp) gave the prediction of actve site and the number of amino acid involved as best active site with volume of 223.5 and area of 203.7. Panel A of the figure 4 shows the probable active site in one-dimension protein sequence whereas lower panels depict the active site residues in three-dimensional space (B and C).

Conclusion

Very little is known about the three-dimensional structure of protein Falstatin from *Plasmodium falciparum* and also it is very difficult to determine structure of proteins experimentally. This lack of information clearly blocks the possibility of transferring available facts of cysteine proteases regulation for development of new anti-malarial drugs. Thus, an in-silico approach is the most efficient way of structural characterization of proteins. Molecular modeling of the PfFalstatin provided us the 3D structures of the protein. Three-dimension structure of the parasite protein could act as a staring material for the in-silico drug screening. Not only that, but the prediction of the active site might also be useful in understanding the enzymatic activity of the protein which is crucial in deciphering the regulation of cysteine proteases. In addition, comparison of modelled PfFalstatin with the structure of cysteine proteases inhibitor from P. berghei, revealed major structural differences. These discrepancies could also fasten the process of drug development against malaria parasites.



[A] **[B]**

Figure 1
Modelled structure of PfFalstatin. A) Ribbon diagram; B) Surface topology

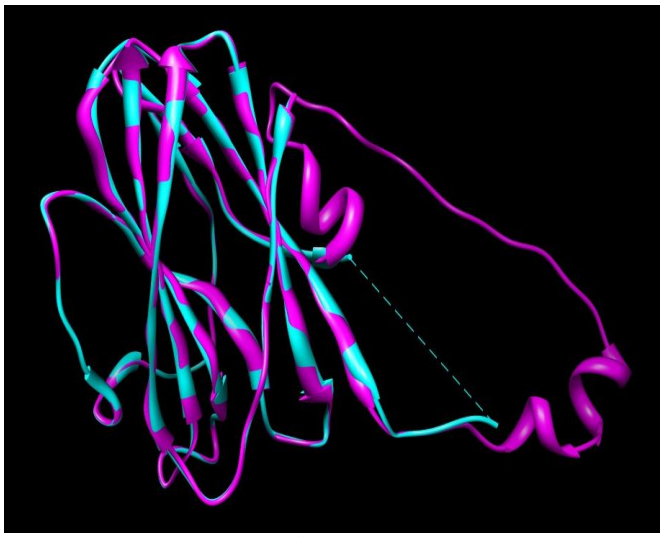


Figure 2
Structural comparison of PfFalstatin with cysteine protease inhibitor of *Plasmodium berghei*

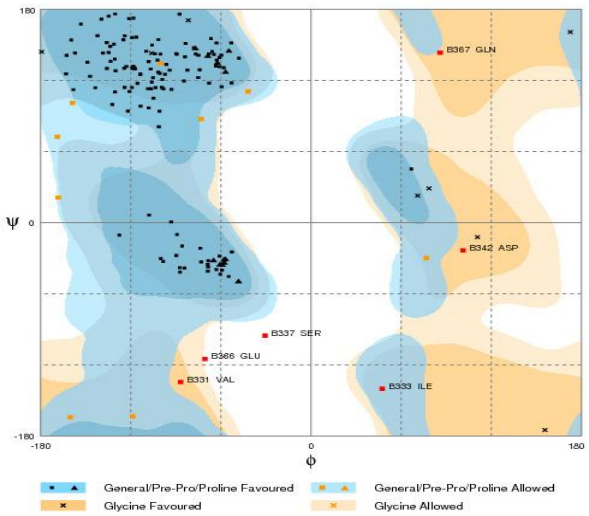
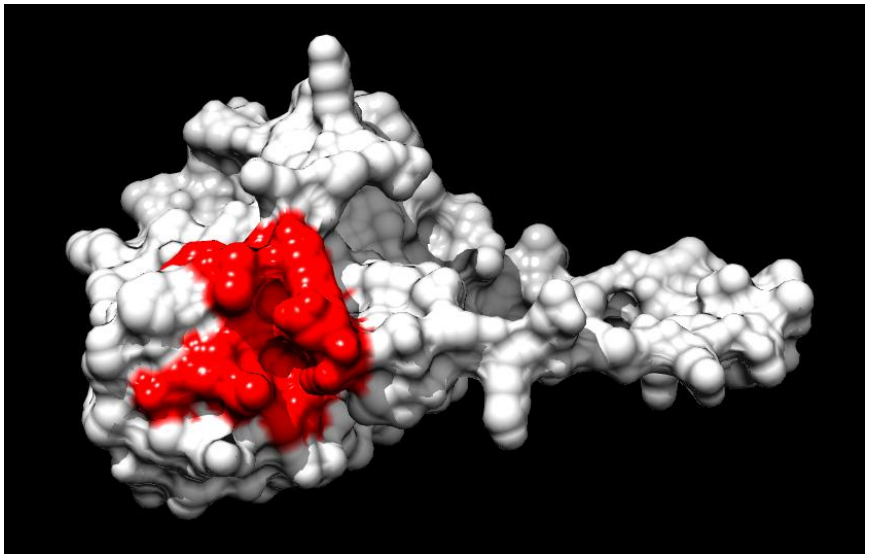


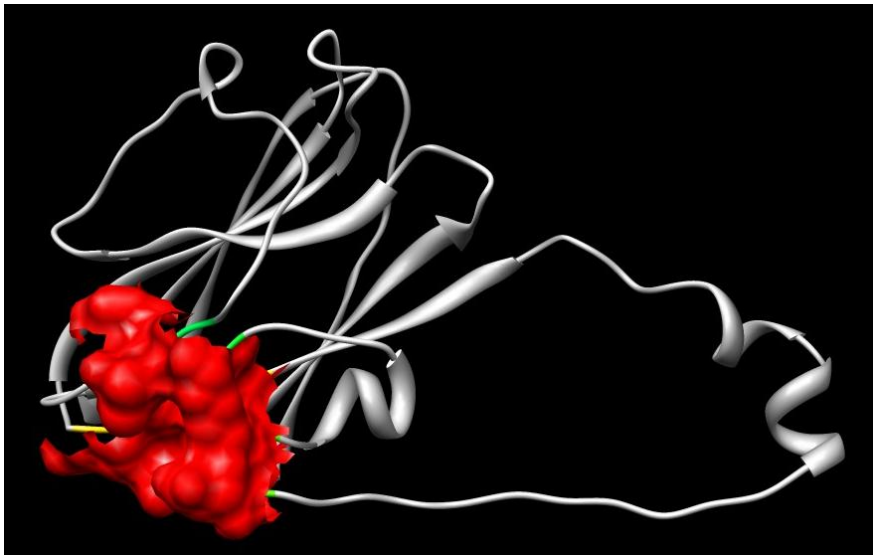
Figure 3
Ramachandran plot of PfFalstatin using Rampage

253- D Q I I K L G D I I N S V N E K I I S I N S T V N N V L C I N L D S V N G N G F V W T L L G V H K K
303- K P L I D P S N F P T K R V T Q S Y V S P D I S V T N P V P I P K N S N T N K D D S I N N K Q D G S
353- Q N N T T T N H F P K P R E Q L V G G S S M L I S K I K P H K P G K Y F I V Y S Y Y R P F D P T R D
403- T N T R I V E L N V Q

A



B



C

Figure 4
Prediction of active site of PfFalstatin using CASTp
A) Active site residues shown in green color
B) and C) Position of active site residues in three-dimensional space

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