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Structural Annotation and Homology Modeling of Protein Kinase 6(PfPK6) from *Plasmodium Falciparum*

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ABSTRACT

Malaria is a major public health burden in the developing world. *Plasmodium falciparum* enters in blood stream and replicates in S-phase of cell cycle and releases merozoites. PfPK6, a putative homologue of p34*cdc* cyclin-dependent kinase (CDK) required for entry into both S-phase and mitosis. Our work suggests that PfPK6 protein can acts as target for the inhibition of *Plasmodium falciparum* activity in Malaria disease. The present investigation includes retrieval of amino acid information and sequence analysis of PfPK6 from major protein sequence database and tools. The Physiochemical parameters like amino acid propensity, molecular weight, isoelectric point, aliphatic index, hydropathicity were determined. The secondary structure of PfPK6 was predicted using SOPMA secondary structure method. The 3D structure was predicted using SWISS-MODEL server and model was further validated using PROCHECK analysis tool. The function of PfPK6 was identified by Pfam domain database. *In silico* structural characterization and homology modeling of PfPK6 can put insight into development of new antimalarial drugs by structure based drug designing.

Keywords: Malaria, *Plasmodium falciparum*, PfPK6, *In silico* , cyclin-dependent kinase etc.

I. INTRODUCTION

Plasmodium falciparum causes the most severe form of malaria and kills up to 2.7 million people annually (1). Despite the global importance of *P. falciparum*, the vast majority of its proteins have not been characterized experimentally. The malaria parasite, *Plasmodium*, is a major public health burden in the developing world and despite the existence of antimalarial treatment active in blood stages of infection, there is a continual need for novel drug design as the parasite develops resistance to current treatments (2). *Plasmodium falciparum*, the causative agent of human malaria, undergoes distinct morphological changes during the progression through its life cycle in the mosquito and human hosts. The parasite forms known as merozoites are released into the bloodstream after the infection of a hepatocyte, invade erythrocytes and mature through several distinct forms (3). Although the correlation between the malaria parasite erythrocytic stages and the classical eukaryotic cell cycle phases is not known, mature trophozoites exhibit peak DNA synthesis and might correspond to S-phase (4). PfPK6, a putative homologue of p34*cdc* cyclin-dependent kinase (CDK) required for entry into both S-phase and mitosis in fission yeast, has been characterized in *P. falciparum* with 60% identity with human cdc2 (5). Cyclin-dependent kinase(CDKs) are essential regulators of the cell cycle. *Plasmodium falciparum* protein kinase 6(PfPK6), a Ser/Thr protein kinase produced by the malaria parasite *Plasmodium falciparum* is an enzyme with homology to human CDKs and the closely related mitogen-activated protein kinase(MAPKs).These significant differences between the plasmodial PfPK6 and its human CDK homologues, make a possible plasmodia-specific anti-malarial therapeutic target(6). Hence the structural characterization and analysis of PfPK6 molecule by *In silico* approach can aid the design and development of novel Cyclin dependent protein kinase inhibitor or anti-malarial theruptic drug target Malaria disease.

II. METHODS AND MATERIAL

1. Retrieval of PfPK6 (*Plasmodium falciparum* protein kinase 6) from Protein database

The protein sequence of *Plasmodium falciparum* protein kinase (PfPK6) was retrieved from UniProtKB Knowledgebase database (7). UniProtKB is public protein database which contains the amino acid sequences of proteins. The sequence was retrieved & saved in FASTA file format with its Accession ID.

2. Primary Sequence Analysis by Protparam tool

Analysis of physiochemical properties of the PfPK6 protein was performed by using ProtParam analysis tool which on ExPASy server [8]. It allows the computation of various physical and chemical parameters for a given protein The computed parameters includes amino acid composition, molecular weight, theoretical pI, Instability index, Grand average of hydropathicity.

3. Secondary Structure Prediction

The secondary structure of PfPK6 was predicted by SOPMA secondary structure prediction method [9]. SOPMA stands for self-optimized prediction method with alignment for the prediction of helix, strands and coils of the protein sequence.

4. Homology Modeling & Validation

The 3D structure of PfPK6 was predicted by using Swiss-model server [10-11]. The selection of template was accomplished by protein BLAST using PDB database having identity more than 30%. The evaluation and validation of generated model was performed with PROCHECK server on PDBSum database [12] and predicted model was visualized by Rasmol visualization tool [13].

5. Identification of functional domain in PfPK6 from Pfam database

The domain identification was carried out by using Pfam database of *Plasmodium falciparum* protein PfPK6. Pfam is a database of protein domain families. Pfam contains curated multiple sequence alignments for each family, as well as profile hidden Markov models (profile HMMs) for finding these domains in new sequences. Pfam contains functional annotation, literature references and database links for each family. [14]

III. RESULTS AND DISCUSSION

1. Retrieval of PfPK6 (*Plasmodium falciparum* protein kinase 6) from Protein database

The PfPK6 sequence from *Plasmodium falciparum* [Uniprot ID: O77239] was retrieved from UniProtKB database, with its 305 amino acids and saved in FASTA format which shown as as below

>tr|O77239|O77239_PLAFA Cdk-related protein kinase 6 OS=Plasmodium falciparum GN=PK6 PE=2 SV=1
MNRIDISNFDFLYVIGKGTYGIVYKALDKKENNFVAIK
KIINLCDENYGISKCILRELTILQKIKHKNIINLKYVFYGK
DIEDKLKGENLENSCLYLAFEYCDIDLFNLIKKHNLNIK
EIKYIIFELLLALSYFHSNNYIHRDIKPENIFITSEGEIKLG
DLGMSVEKSDHMTPTVVTLWYRAPEILLKSTNYDQKV
DIWSLGCLFMELIQGRPLFPGKNDCTQLELIYLLLGDKD
KLTTVDKERKDMFPYFEINMLKDAIDDEHTLDLISKML
IYDPNYRISSKEALKHPCFQDIEQVKFSYNF

2. Primary Sequence Analysis by Protparam tool

Physicochemical composition of PfPK6 was analyzed by using ProtParam analysis tool which on Expasy server. The physiochemical parameters were tabulated in Table 1. As per the table instability index is 26.21 classifies the PfPK6 is stable, on the basis theoretical pI the PfPK6 is acidic in nature. The negatively charged residues are more than positively charged residues. Cdk-related protein kinase 6 protein contains 305 amino acids with molecular weight 35843.69 dalton.

Table 1. Physicochemical Properties of PfPK6 protein

Parameters	Values
Molecular weight	35843.69
Theoretical pi	5.87
Instability index	26.21
Extinction coefficients	38195
Total number of negatively charged residues (Asp + Glu)	45
Total number of positively charged residues (Arg +	40

Lys)	
Aliphatic index	105.15
GRAVY	-0.212

Secondary Structure Prediction

The secondary structure of the PfPK6 was predicted using SOPMA server. The percentage of secondary structure elements was tabulated in Table 2 and graphical representation which shown in Figure 1. It was observed that alpha helix was predominant (35.08%), followed by random coil (29.18%)and extended strand (24.26%). Alpha helix serve as the major secondary structural elements for a protein molecule and play a major role in structural stability of protein.

Table 2. Secondary structure of PfPK6 protein using SOPMA

Parameter	Values
Alpha helix	35.08%
Extended strands	24.26%
Coils	29.18%

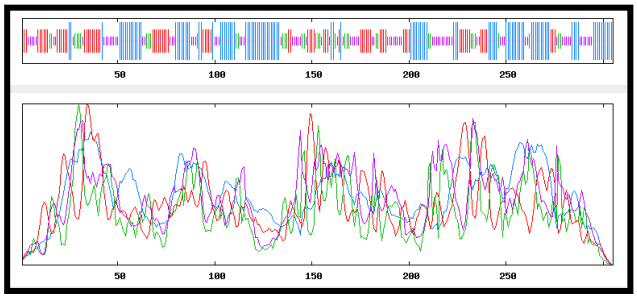

Figure 1. Secondary structure of PfPK6 protein using SOPMA

4. Homology modeling and model evaluation

The SWISS-MODEL homology modeling program was used for the predicting of three dimensional structure of the PfPK6 protein which shown in Figure 2.The Cyclin dependent kinase 13(PDB ID: 5EFQ) was selected as template with 38.62% sequence identity to query sequence. The quality and validation of the model was further evaluated by Ramachandran plot analysis using PDBsumserver which shown in Figure 3. Ramachandran plot analysis showed that only 1.8% residues in outlier region, 5.1% allowed region and **93.0%** in favored region, indicating that the models were of reliable and good quality.

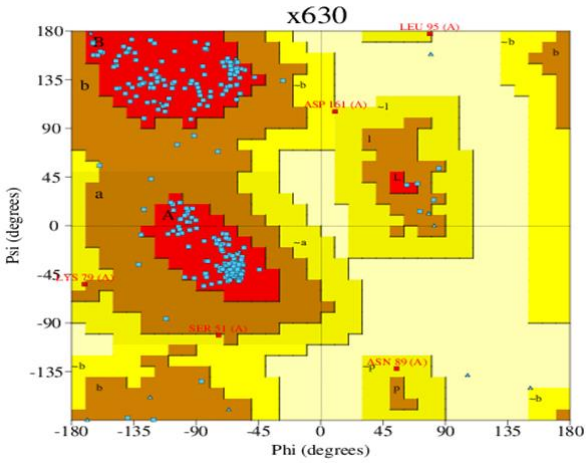

Figure 2. PROCHECK analysis

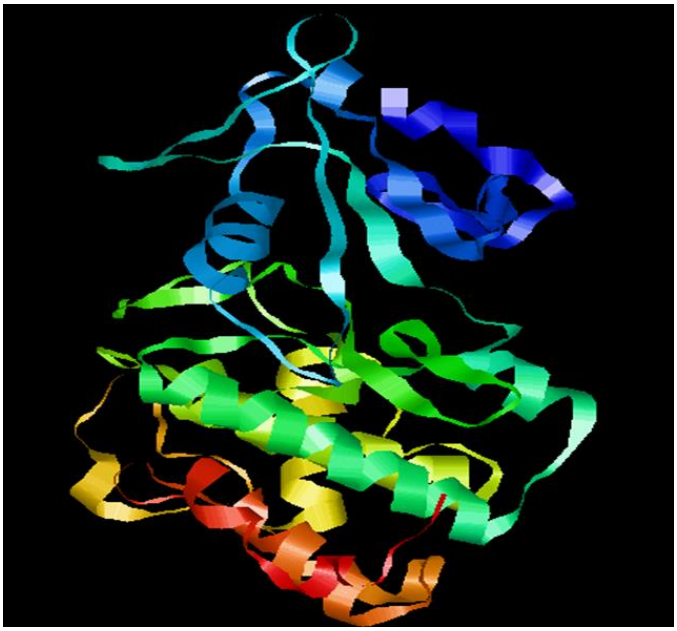

Figure 3. 3D Structure of PfPK6

5. Identification of functional domain in PfPK6 from Pfam database

The domain identification of PfPK6 from *Plasmodium falciparum* predicted by Pfam domain database. The domain structure was shown in Figure 4.The PfPK6 belongs from Protein kinases family which play a role in a multitude of cellular processes, including division, proliferation, apoptosis, and differentiation. Phosphorylation usually results in a functional change of

the target protein by changing enzyme activity, cellular location, or association with other proteins.

Figure 4. Functional Domain structure

IV. CONCLUSION

The PfPK6(*Plasmodium falciparum* protein kinase 6) enzyme involved in the S-phase and mitosis process of *Plasmodium falciparum* causing malaria disease. In present study, we analyzed the physicochemical properties of PfPK6 protein by using Protparam tool. The secondary structural elements were predicted by SOPMA tool. The 3D structure of PfPK6 was predicted using SWISS MODEL server and the final model was further evaluated by using PROCHECK and Ramachandran plot analysis which shown 93.0% reliability. The PfPK6 belongs to Protein kinase family by Pfam functional domain database. The structural characterization and homology modeling shows that PfPK6 can be used as target in malaria disease. The molecular and structural insights encompasses the development of new antimalarial drugs for inhibition of PfPK6 (*Plasmodium falciparum* protein kinase 6) by structure based drug designing approach.

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