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In-Silico Molecular Characterization of PvDBP Involved in Malaria

Vinod P. Sinoorkar[1], Shruti S. Kalashetti[2]* and Deepali S. Pawar[2]
[1]PG Department of Bioinformatics, Walchand College of Arts & Science , Solapur, Maharashtra, India
[2]Department of Zoology, Walchand College of Arts & Science ,Solapur, Maharashtra, India

ABSTRACT

Malaria is a major public health problem in India and one which contributes significantly to the overall malaria burden in Southeast Asia. India recorded 6% of the world's new malaria cases in 2016 which stood at 216 million according to the World Malaria report 2017 by World Health Organization (WHO). India witnessed a total of 331 malaria death in 2016 making it the highest in the entire Southeast Asia region. The two major human malaria parasite species in India are Plasmodium falciparum and *P.* vivax, P. malarie has been reported in the eastern India. Understanding the pathogenesis of malaria requires investigation of mechanisms including parasite invasion, parasite biology and host defence. The parasite life cycle illustrates the interplay of parasite and host interactions. It is during the blood-stage of infection that malaria disease occurs and host immune responses to merozoite surface antigens and targeting merozoite surface proteins and invasion ligands have been important areas of research. *P.* vivax has a very strong preference for invasion of reticulocytes and is largely restricted to invading young reticulocytes that still bear CD71. Plasmodium vivax requires interaction with the Duffy antigen receptor for chemokines (DARC) to enable its invasion of human erythrocytes. Interaction with DARC is mediated by the *P. vivax* Duffy-binding protein (PvDBP) and is essential for junction formation, which is a key step in the invasion process. Hence our present study mainly focuses to unfold the molecular mechanism of PvDBP in invasion process by detailed understanding of its molecular features viz retrieving of protein sequence information, analysis of physicochemical properties, secondary structural elements, prediction of tertiary structure, analysis of binding domains and residues using various bioinformatics tools and softwares. Thorough understanding of molecular structure and function of PvDBP can put insights into its key role in invasion process and can serve as major protein target for structure based drug designing against malaria.

Keywords : Plasmodium vivax, invasion, reticulocyte, Duffy binding protein, drug designing.

I. INTRODUCTION

Malaria is a major public health problem in India and one which contributes significantly to the overall malaria burden in Southeast Asia. India recorded 6% of world's new malaria cases in 2016 which stood at 216 million according to the world malaria report 2017 by world health organization (WHO).India witnessed a total of 331 malaria death in 2016 making it the highest in the entire Southeast Asia region. The two major human malaria species in India are *Plasmodium falciparum* and *P. Vivax, P.malarie* has been reported in the eastern India (Sharma *et al.*, 2006), the two major infecting species vary in proportion across India. *P. falciparum* and *P. vivax* are unevenly distributed across India. The ratio

of *P.falciparum* versus *P. Vivax* malaria gradually increasing to 0.60 by 1995, and shifting to 1.01 by 2010 (Singh *et al.,* 2004a,b). Pathogenesis results from rupture of infected red blood cells, leading to fever. Understanding the pathogenesis of malaria requires investigation of mechanisms including parasite invasion, parasite biology, and host defence. The parasite life cycle illustrates the interplay of parasite and host interactions (DANNY A Milner, Jr, MD, MSc (Epi)) *Plasmodium vivax* requires two hosts to complete its life cycle. In human body the parasite multiplies asexually while in female anopheles it undergoes a sexual cycle followed by an asexual multiplication called sporogony. The trophozoite phase of plasmodium occurs in RBCs of human beings. The parasite first invades the liver cells for asexual multiplication. The schizont divides by multiple fission to from 6-24 daughter nuclei which migrate towards the periphery and bursts liberating the merozoites.

Merozoite form of the *p.vivax* invades red blood cells and replicates inside them. It is during the blood-stage of infection that malaria disease occurs and host immune responses to merozoite surface antigens, and targeting merozoite surface proteins and invasion ligands by novel vaccines and therapeutics have been important areas of research. Merozoite invasion involves multiple interactions and events, and substantial processing of merozoite surface proteins occurs. The merozoite surface is highly complex, presenting a multitude of antigens to the immune system. This complexity has proved challenging to our efforts to understand merozoite invasion and malaria immunity.

*P.vi*vax has a very strong preference for invasion of reticulocytes and is largely restricted to invading young reticulocytes that still bear CD71 (Malleret *et al.* 2015). In addition, efficient invasion by *P. vivax* requires the Duffy antigen on reticulocytes, although invasion into Duffy-negative reticulocytes can occur. (Ryan *et al.* 2006).

II. METHODOLOGY

1.Protein sequence retrieval

Protein sequence data of Duffy Binding Protein has been retrieved by UNIPROT and saved in fasta format with the Uniprot ID (http://expasy.org/uniprot/)

2. Analysis of physico-chemical properties

Physico-chemical properties of PvDBP were analysed by using protparam tool available at Expasy server (http://web.expasy.org/protparam/)

3. Secondary structure analysis

The secondary structure of Duffy Binding Protein was predicted by SOPMA it was employed for calculating the secondary structured feature.(http://web.expasy.org/sopma).

4. Prediction of antigenicity

Prediction of major antigenic regions in PvDBP was carried out by using Kolaskar and Tongaonkar antigenicity prediction method.

5. Identification of domain

The conserved domain of Duffy Binding Protein was analysed by using Pfam data base [protein family]. (http://pfam.xfarm.org)

6. Homologgy modelling and validation

The 3D structure of Duffy Binding Protein was generated by this server. The PROCHECK analyses provide an idea of the stereo chemical quality of all protein chains in a given PDB structure by Roman Zoskow ki; 1992.

7. Structure visualization

The predicted 3D structure of both Duffy Binding Protein and Duffy Antigen was visualized by using Raswin.

8. Prediction of binding pockets

The potent binding cavities of PvDBP where identified by using Vlife Biopedicta Tool.The most potent cavity with highest hydrophobicity was displaced.

III. RESULTS AND DISCUSSION

1. Retrieval of protein sequence information of PvDBP

For the study of PvDBP molecule, its amino acid sequence was retrieved from the major protein sequence databases like UniprotKB and NCBI protein database. The sequence obtained was stored in Fasta format with its accession number. The length of the sequence was found to be 487 aa as shown in figure 1.

Accession no.- Q3Y5W2

>tr|Q3Y5W2|Q3Y5W2_PLAVI Duffy binding protein (Fragment) OS=Plasmodium vivax OX=5855 PE=4 SV=1

MKGKNRSLFVLLVLLLSHKVNNVLLERTIETLLECK
NEVKGENGYKLAKGHHCVEEDNLERWLQGTNER
RSEENIKYKYGVTELKIKYAQMNGKRSSRILKESIYG
AHNFGGNSEGKDGGDKTGEEKDGEHKTDSKTDNG
KGANNLVMLDYETSSNGQPAGTLDNVLEFVTGHE
GNSRKNSSNGGNPYDIKTISSAIINHAFLQNTVMKN
CNYKRKRRERDWDCNTKDVCIPDRRYQLCMKELT
NLVNNTDTNFHSDITFRKLYLKRKLIYDAAVEGDLL
LKLNNYRYNKDFCKDIRWSLGDFGDIIMGTDMEGI
GYSKVVENNLRSIFGTGKNAQQHRKQWWNESKA
QIWTAMMYSVKKRLKGKFIWICKINVAVNIEPQIY
RRIREWGRDYVSELPTEVQKLKEKCDGKINYTDKK
VCKVPPCQNACKSYDQWITRKKNQWDVLSNKFKS
VKNAEKVQTAGIVTPYDILKQELDEFNEVAFENEIN
KRDGAYIELCVCSVEEAKKNTQEVVTNVDNAAKS
QATNSNPISQPVDSSKAEKVPGDSTHGNVNSGQDS
STTGKAVTGDGQNGNQTPAESDVQRSDIAESVSAK
NVDPQKSVSKRSDDTASVTGIAEAGKENLGASNSR
PSESTVEANSPGDDTVNSASIPVVSGENPLVTPYNG
LRHSKDNSDSDGPAESMANPDSNSKGETGKGQDN
DMAKATKDSSNSSDGTSSATGDTTDAVDREINKGV
PEDRDKTVGSKDGGGEDNSANKDAATVVGEDRIR
ENSVGNSTNDRSKNDTEKNGASTPDSKQSEDATAL
SKTESLESTESGDRTTNDTTNSLENKNGGKEKDLQK
HDFKSNDTPNEEPNSDQTTDAEGHDRDSIKNDKAE
RRKHMNKDTFTKNTNSHHLNSNNNLSNGKLDIKE
YKYRDVKATREDIILMSSVRKCNNNISLEYCNSVED
KISSNTCSREKSKNLCCSISDFCLNYFDVNSYEYHSC
MKKEFEDPSYKCFTKGGFKDKTYFAAAGALLILLLL
IASRKMIKNDSEEATFNEFEEYCDNIHRIPLM

Figure 1. Showing Protein sequence of PvDBP

2 Analysis of Physicochemical properties

The analysis of physicochemical properties of PvDBP was done by using protein prediction tool i.e. protparam which gives a detailed information of protein i.e. isoelectrical point, theoretical pI, extension coefficient, half life, instability index, Aliphatic index, Grand average, hydropathicity, etc., were predicted, calculated and tabulated as shown in table 1.

Table 1. Showing Physicochemical properties of PvDBP

Properties	Values
No. of amino acid	1054
Molecular weight	117729.99
Theoretical Pi	6.07
Total no. of negative charged residue (Asp+Glu)	165
Total no .of positively charged residue (Arg +Lys)	154
Atomic composition 1)Carbon(C)	5020
2)Hydrogen(H)	7996
3)Nitrogen(N)	1478
4)Oxygen(o)	1712
5)Sulphur(s)	40
Total no .of atoms	16246
Extinction coefficient No.	105545
Half life	30hrs

Instability index	34.55(stable)
Aliphatic index	60.77
Grand average of a hydropathicity	-0.980

3. Secondary structure prediction of PvDBP

Secondary structure prediction of PvDBP was carried out by using SOPMA and all the secondary structural elements like alpha helix, beta sheets, random coils and extended strands were predicted as shown in table 2. (Gobel, 1994)

Table 2. Showing secondary structure information of PvDBP

Structural component	Residues	Percentage
Alpha helix (Hh)	359	34.06%
3_{10} helix (Gg)	0	0.00%
Pi helix (Ii)	0	0.00%
Beta bridge (Bb)	0	0.00%
Extended strand(Ee)	142	13.47%
Beta turn (Tt)	71	6.74%
Bend region(Ss)	0	0.00%
Random coil (Cc)	482	45.73%
Ambiguous	0	0.0%

4. Prediction Tertiary structure of PvDBP

The Tertiary structure of PvDBP was obtained by using SWISS Model tool by selecting the template with maximum homology and with optimised parameters. The obtained structure was stored in pdb format for visualization. The details of template selected for structure prediction was as shown on table 3.

Table 3. Showing template information of PvDBP for homology modelling

Name	Title	Identity	Oligostate
4yfs.1.A	Duffy receptor	95.99	Monomer
3rrc.1.A	Duffy receptor	97.14	homo-dimer
3rrc.1.B	Duffy receptor	97.14	homo-dimer
5f3j.2.A	Duffy receptor	97.14	Hetero-oligomer
4nuv.2.A	Duffy receptor	97.14	Hetero-oligomer

5. Prediction of antigenicity

Prediction of major antigenic regions in PvDBP was carried out by using Kolaskar and Tongaonkar antigenicity prediction method as shown in table no.4

Table 4. showing potent antigenic regions

No	Start	End	Peptide	Length
1	7	27	SLFVLLVLLLS HKVNNVLLER	21
2	267	291	FRKLYLKRKLI YDAAVEGDLLLKLN	25
3	371	391	GKFIWICKIN VAVNIEPQIYR	21
4	423	441	DKKVCKVPP CQNACKSYDQ	19
5	593	610	AESVSAKNV DPQKSVSKR	18
6	651	668	NSASIPVVS GENPLVTPY	18
7	962	988	KNLCCSISDFCLNY FDVNSYEYHSCMK	27
8	1007	1026	KTYFAAAGAL LILLLLIASR	20

6. Visualization of tertiary structure of PvDBP

The predicted Tertiary structure of PvDBP was visualized by using structure visualization tool Rasmol. Visualization was done using different models and formats to understand structural features of PvDBP. The various models were represented as shown in figure 2. (Gettins PG, 2002)

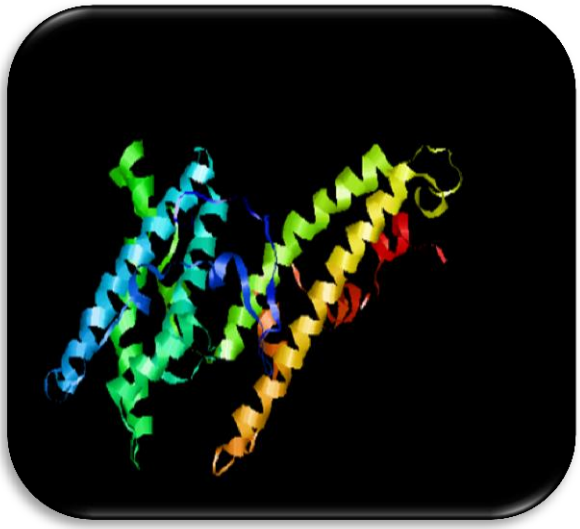

Figure 2. 3D ribbon structure of PvDBP in Rasmol

7. Prediction of domains

The conserved domain of Duffy Binding Protein was analysed by using Pfam data base [protein family]. (http://pfam.xfarm.org) and the conserved domains are as shown in figure 3.

Figure 3. conserved domains of PvDBP

8. Prediction of Binding Pockets

The potent binding cavities of PvDBP where identified by using Vlife Biopedicta tool. The most potent cavity with highest hydrophobicity was displaced as shown in figure 4.

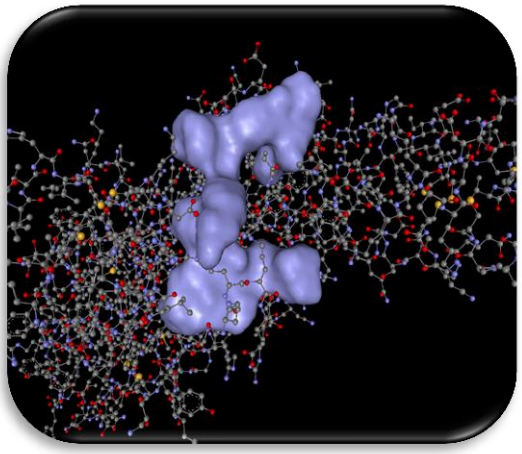

Figure 3. potent binding pocket of PvDBP

IV. CONCLUSION

- Malaria is a major public health problem in India and one which contributes significantly to the overall malaria burden in Southeast Asia.
- India witnessed a total of 331 malaria death in 2016 making it the highest in the entire Southeast Asia region.
- The two major human malaria species in India are Plasmodium falciparum and P. Vivax has been reported in the eastern India.
- Plasmodium vivax requires two hosts to complete its life cycle i.e human body & female anopheles mosquito.
- P. vivax has a very strong preference for invasion of reticulocytes and is largely restricted to invading young reticulocytes that still bear CD71.
- Plasmodium vivax reqires interaction with DARC is mediated by the P. vivax Duffy-binding protein (PvDBP) and is essential for junction
  formation, which is a key step in the invasion process.
- Knowing the key role of PvDBP in malaria, the present study was conducted to understand the molecular basis of PvDBP by using its sequence information, deducing various physico-chemical properties, predicting

secondary structural elements, modeling the tertiary structure, finding the possible antigenic regions, conserved domains and identifying the potent binding pockets of PvDBP.

- Further detailed understanding of binding and interaction of PvDBP with human host, can serve as new approach to treat malaria through structure based drug designing.

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