



Research Article

Computational analysis, structural modeling and ligand binding site prediction of *Plasmodium falciparum* 1-deoxy-D-xylulose-5-phosphate synthase



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ABSTRACT

Malaria remains one of the most serious infectious diseases in the world. There are five human species of the *Plasmodium* genus, of which *Plasmodium falciparum* is the most virulent and responsible for the vast majority of malaria related deaths. The unique biochemical processes that exist in *Plasmodium falciparum* provide a useful way to develop novel inhibitors. One such biochemical pathway is the methyl erythritol phosphate pathway (MEP), required to synthesize isoprenoid precursors. In the present study, a detailed computational analysis has been performed for 1-deoxy-D-xylulose-5-phosphate synthase, a key enzyme in MEP. The protein is found to be stable and residues from 825 to 971 are highly conserved across species. The homology model of the enzyme is developed using three web-based servers and Modeller software. It has twelve disordered regions indicating its druggability. Virtual screening of ZINC database identifies ten potential compounds in thiamine diphosphate binding region of the enzyme.

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1. Introduction

Malaria is one of the most serious infectious diseases in the world as evident from the 2016 World Health Organization report, released in December 2015, “there were 214 million cases of malaria in 2015 and 438,000 deaths” (World Health Organization, 2016). Malaria also imposes a huge economic burden mostly on developing countries and it has been estimated that it reduces the economic growth by approximately 1.3% each year (Gallup and Sachs, 2001). The most responsible and virulent species of the *Plasmodium* genus for causing malaria is *Plasmodium falciparum* (Rich et al., 2009; Christopher et al., 2012). The rapid development of resistance to chemotherapeutic agents, often multidrug resistance, were observed in *P. falciparum* (Greenwood and Muta-bingwa, 2002; Ines et al., 2011; Daniel et al., 2012) posing a challenge for future drug development programme. Thus, it is crucial to find out new molecular targets and new classes of drugs to overcome the issue of drug resistance in *P. falciparum*. One vital or plausible target may be the unique biochemical processes that exist in *P. falciparum* and not in humans.

In *P. falciparum* such unique biochemical reactions occur inside the apicoplast, a non-photosynthetic plastid (Cilingir and Broschat,

2012). One such unique targetable pathway is the methyl erythritol phosphate (MEP) pathway for isoprenoid precursor biosynthesis (Gräwert et al., 2011; Masini and Hirsch, 2014) as it is present in all intra-erythrocytic stages of the parasite (Stuart et al., 2004; van der Meer and Hirsch, 2012).

The first reaction in MEP pathway is the condensation of glyceraldehyde-3-phosphate and pyruvate to form 1-deoxy-D-xylulose-5-phosphate (DXP). Again, DXP is also essential for vitamin B1 and B6 biosynthesis (Lois et al., 1998). The reaction is catalyzed by 1-deoxy-D-xylulose-5-phosphate synthase (DXP synthase), which has a thiamine binding motif and requires thiamine diphosphate (ThDP) and either Mn^{2+} or Mg^{2+} for its activity (Kuzuyama et al., 2000). Thus, DXP synthase becomes a possible target for the development of antimalarial drugs. To get insight into the structural features of *P. falciparum* DXP synthase, a crystal structure or NMR structure would be valuable. Still to date the only crystal structures available for this enzyme are from *E. coli* and *D. radiodurans* (Xiang et al., 2007). Recently a homology model of *M. tuberculosis* DXP synthase has been reported with the potential screening of inhibitors (Masini et al., 2015).

So in the present study, I have done computational analysis to determine the chemical and structural properties of *P. falciparum* DXP synthase, along with its protein–protein interaction network. Due to the lack of three dimensional and structural information of the enzyme, I have taken a comparative homology based modeling

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techniques to built its structure. The best model is used for further prediction and analysis of its ligand-binding sites and potentially druggable cavities. I have also done virtual screening of ZINC database for finding possible chemical compounds for future drug designing.

2. Materials and methods

2.1. Sequence retrieval

The amino acid sequences of DXP synthase of *P. falciparum* 3D7 (Accession no. XP_001350100.1) were retrieved from National Center for Biotechnology Information (NCBI). The protein is 1205 amino acids long and used for further analysis in the current study.

2.2. Primary and secondary structure details

ExPasy's ProtParam tool (Colovos and Yeates, 1993) was utilized to calculate the physico-chemical properties of DXP synthase. The theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient (Gill and Von, 1989), instability index (Guruprasad et al., 1990), aliphatic index (Ikai, 1980) and grand average hydropathicity (GRAVY) of the protein were calculated using the default parameters. Secondary structure of DXP synthase was predicted by using SOPMA (Guermeur et al., 1999) and PSIPRED Server (Buchan et al., 2013). The server also predicted secondary structural properties such as α helix, 3_{10} helix, Pi helix, Beta Bridge, Extended strand, Bend region, Beta turns, Random coil, Ambiguous states and other states.

2.3. Evolutionary conservation analysis

Evolutionary conservation analysis of DXP synthase was performed using the ConSurf server (<http://consurf.tau.ac.il/>) (Ashkenazy et al., 2010). It estimated the evolutionary conservation of amino acid positions in a protein based on the phylogenetic relations between homologous sequences and generated a color-based conservation score for *P. falciparum* DXP synthase to reveal the degree of conservation.

2.4. Disordered region prediction

GlobPlot 2.3 (<http://globplot.embl.de/>) was used for exploring disorder and globular segments of DXP synthase (Linding et al., 2003).

2.5. Network interaction

STRING (Snel et al., 2000) was used to identify protein–protein interaction partners of DXP synthase selecting the *P. falciparum* in the “Organism” tab drop down list.

2.6. Homology modeling of DXP synthase

Homology modeling of *P. falciparum* DXP synthase was carried out to predict its three dimensional (3D) structure. Three web-based servers like HHPred (Biegert et al., 2006), RaptorX (Källberg et al., 2012) and (PS)2 (Huang et al., 2015) and one software, Modeller (v 9.13) (Fiser and Sali, 2003) are used for homology modeling of DXP synthase. The steps, for using the web servers for prediction of homology based model of DXP synthase, are like a previous study (Goswami, 2015). The HHPred server predicts the atomic coordinates using MODELLER v. 9.2 (Fiser and Sali, 2003). The updated (PS)2 server (<http://ps2v3.life.nctu.edu.tw/>) was used to build homologous 3D structures of *P. falciparum* DXP synthase.

The activity of the server is based on effective consensus strategy in both template selection and target–template alignment to generate the modeled structure. For Modeller twenty five structures are generated and the best structure has been chosen based on DOPE score and GA341 score. The predicted 3D structure of DXP synthase was visualized by PyMOL (<http://www.pymol.org/>) The obtained structural models of DXP synthase from the web servers and Modeller were then refined by GALAXY Refine server (Heo et al., 2013) and then subjected to molecular dynamic simulation in MDweb (Hospital et al., 2012) using Gromacs full MD setup and Amber-99SB* force field.

2.7. Energy minimization of modeled structures of DXP synthase

To improve the quality and distorted geometries of the predicted model of DXP synthase, energy minimization was performed in DeepView v4.04 tool (Guex and Peitsch, 1997) with the GROMOS 96 force field (Van Gunsteren et al., 1996).

2.8. Model validation for DXP synthase

The predicted model of DXP synthase was validated by RAMPAGE (Lovell et al., 2002) and QMEAN (Qualitative Model Energy Analysis) (Benkert et al., 2009) servers. The RAMPAGE server assessed the stereochemical quality of a protein structure and generated Ramachandran plot of the backbone structure.

2.9. Prediction of ligand binding site or cavities of DXP synthase

Protein-ligand binding prediction server COACH (<http://zhanglab.ccmb.med.umich.edu/COACH/>) has been used for the present study to find the possible ligand binding site or cavities of DXP synthase. COACH is a meta-server for protein-ligand binding site prediction (Yang et al., 2013). The energy-minimized and refined structures of DXP synthase, obtained from RaptorX and Modeller were used for the predictions with COACH. These predictions were also combined with results from other methods including COFACTOR (Roy et al., 2012), FINDSITE (Brylinski and Skolnick, 2008) and ConCavity (Capra et al., 2009) to predict ligand binding sites of DXP synthase (Yang et al., 2013).

2.10. Virtual screening of ZINC data base

A structure based virtual screening was performed in a web-based server, idock (Li et al., 2012), to screen chemical compounds from ZINC database (Irwin and Shoichet, 2005). Initially the available crystal structures of *E. coli* DXP synthase (i.e. 2O1S) has been screened for finding potential chemical compounds in idock. Then top 10 ligands are selected (having mol2 file in the ZINC database) on the basis of free energy change. These ligands are then docked with the *P. falciparum* DXP synthase in Swiss Dock web-server (Grosdidier et al., 2011). The results of the docking in SwissDock are opened in UCSF chimera for further analysis (Pettersen et al., 2004).

3. Results and discussion

3.1. Primary and secondary structure analysis

It was important to analyze the protein sequence or the primary structure of the protein as these parameters provide the basic information of the protein stability and function. In this study, the ProtParam tool of the ExPasy server was used for physico-chemical analysis of the *P. falciparum* DXP synthase. This reveals that the protein has a theoretical pI of 8.87, molecular mass of 140,230, aliphatic index of 85.08 and theoretical extinction coefficient (at

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