Comparative modeling of 3-oxoacyl-acyl-carrier protein synthase I/II in *Plasmodium falciparum*– A potent target of malaria

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Abstract- *Plasmodium falciparum* causing malaria is yet reigning against drug design community when it comes to survival and defense. Continuous evolution and drug resistant character is foremost basis of parasite's versatility. 3-oxoacyl-acyl-carrier protein synthase I/II in *Plasmodium falciparum* is discovered decisive in fatty acid synthesis machinery. Objectives of enzyme inhibition need structural characterization from its 3D structure. In present studies molecular modeling of 3-oxoacyl-acyl-carrier protein synthase I/II is achieved using *in silico* comparative modeling. ICM Molsoft algorithm was adopted for comparative modeling which provides an accurate and efficient module to build loops and side chains found non-identical in sequence. Energy parameters fell in thermodynamically stability zone. Modeled structure revealed appreciable measures when validated. Ramachandran plot signified the present work undertaken through conformational parameters Φ (phi) and Ψ (psi) angles calculated from model with 83.2% residues in most favoured region. Further PROCHECK results confirmed acceptance of model through main and side-chain values. Root mean square distance of planarity found below 0.01. Beside some bad contacts, bond angles and bond lengths confer qualitative part of work. Structure of 3-oxoacyl-acyl-carrier protein synthase I/II can be important tool for structure based drug designing techniques to impel the search of new efficient inhibitors. Comparison of similar structures of parasite can further reveal mutational trends to study their evolution patterns.

Keywords- Comparative modeling, Fatty acid synthesis, ICM Molsoft, PROCHECK

Introduction

3-oxoacyl-acyl-carrier protein synthase I/II in *Plasmodium falciparum* belongs to Beta-ketoacyl-ACP synthase I/II family. *Plasmodium falciparum* accounted for 90% of available human malarial infections in 2006 all over the globe including highest contribution in mortality profiles [1]. Every year 1-2 million deaths and 300 to 500 million clinical infections are claimed by this infection and considered as world's most important infectious diseases in terms of mortality [2]. The continuous evolution and drug resistance of strain from this parasite has set need of new chemotherapeutic agents for this infection [3]. Adoption of Structure based drug design requires 3D structure of 3-oxoacyl-acyl-carrier protein synthase I/II from amino acid sequence obtained in *Plasmodium falciparum*. Fatty acid synthase (FAS) found in critical role towards fatty acid synthesis process in Plasmodium species to produce precursors to perpetuate and infect the host cells [4-5]. The discovery of Type II Fatty Acid Synthase (FAS) machinery in the apicoplast for the survival has been supported and distinguished *Plasmodium falciparum* from other species after concluding ability to synthesis fatty acids in its own cells [6]. This fatty acid synthesis route includes role of acyl carrier protein (ACP), β-ketoacyl-ACP synthases III (FabH) and I/II (FabB/F) and enoyl-ACP reductase (FabI) [7-8]. Present work is an *in silico* approach to model structure of 3-oxoacyl-acyl-carrier protein synthase I/II using comparative modeling. Computational modeling has grown with a faster rate to overcome the difference between protein sequences available and structures determined using spectroscopic techniques. This technique has added high levels of understandings in structural studies to functional role of protein involved in efforts to improve human health. Several algorithms have been elaborated by computational biologist approaching efficient modeling although protein having domain variants when treated by same algorithm shows some degrees of impotency of algorithm. At the end of structure validation brings final appraisal in modeling efforts.

Sequence Retrieval

Amino acid sequences retrieved from swissprot/uniprot, provided descriptions of a nonredundant set of proteins including their function, domain structure, posttranslational modifications and variants [9-10]. 3-oxoacyl-acyl-carrier protein synthase I/II sequence from *Plasmodium falciparum* is retrieved as query sequence with total length of 473 amino acids and molecular weight of 52656.5. This database merges all proteins in single entry coded by one gene so as to minimize redundancy and improve reliability with fully featured information. Cross-references with others databases modemize swissprot entries to hold detailed expertise [11].

Template for Modeling

Standalone blastp search was performed for finding similar structure entry in PDB database from ftp download available on ftp://ftp.ncbi.nih.gov/blast/db/FASTA/pdbaa.gz. Results from blastp show 45% identity and 65% similarity with above query from which is a structure of Beta ketoacyl acyl carrier Protein synthase II (Kasii) from *Synechocystis Sp.* 1E5M is PDB entry ID of template which is a X-ray crystallized structure at 1.54 Ang. and selected for backbone alignment with two domains identified in secondary structure studies determined by Moche, M. et al available on http://www.rcsb.org/pdb/explore/explore.do?structureId=1E5M.

Comparative Modeling

For modeling beta-ketoacyl- ACP synthase I/II structure ICM Molsoft has been adopted. ICM Molsoft algorithm contains robust modeling tools and high levels of accuracy with fast model building [12-14]. After initial alignment of query polypeptide chain on template backbone, side chain torsion angles are optimized using simultaneous global optimization of the energy for all non-identical residues. ICM Biased Probability Monte Carlo (BPMC) optimization facilitates conformational modeling through defining internal coordinates [15]. Extensive force field terms are conjoined and side entropy parameters proved ICM towards accuracy acceptance in CASP2 protein modeling competition [16-19]. Sequence of 3-oxoacyl-acyl-carrier protein synthase I/II is aligned on backbone of 1E5M structure and comparative modeling was performed in ICM Molsoft. Two Loops having RMSD values 0.1 are identified and modeled using PDB loop database using local energy minimization protocols in ICM. Energy minimization for modeled structure thermodynamically proved accepted structure with energy of -19317.473 KJ/mol. Modeled structure of 3-oxoacyl-acyl-carrier protein synthase I/II is shown in figure-1. Ramachandran plot analysis presented in figure-2.

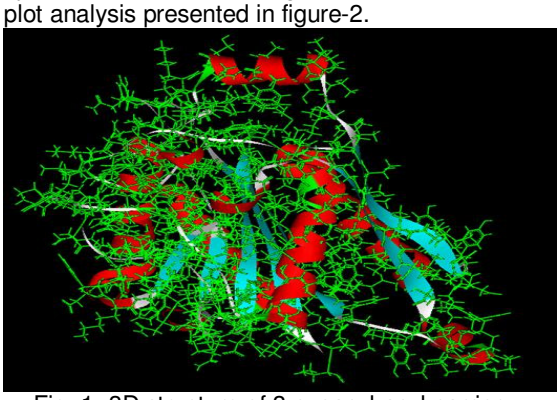

Fig. 1- 3D structure of 3-oxoacyl-acyl-carrier protein synthase I/II

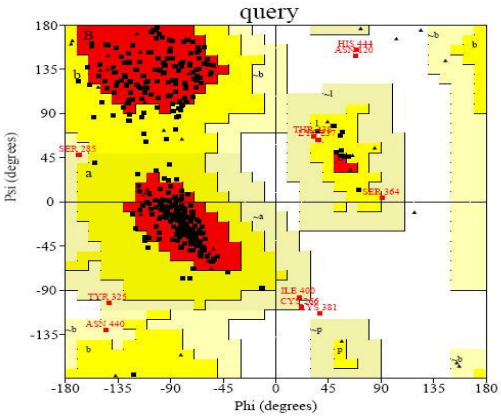

Fig. 2- Ramachandran Plot of model

Structure Validation using PROCHECK

Structure validation of enzyme structure modeled above is processed using PROCHECK which determine stereochemical aspects along with main chain and side chain parameters with comprehensive analysis [20-21]. Optimization and analysis of bond length and bond angles is referred from Cambridge Structural Database, CSD after studying 100,000 structures [22]. PROCHECK analysis reveals in Ramachandran plot concluding Phi and psi angles to contribute in conformations of amino acids excluding glycine and proline with 83.2% in (298) residues in most favored region, 13.7%(49) in additional allowed region 2.0%(7) in generously allowed region and 1.1%(4) residues in disallowed region. Although standards allow model acceptance in 90% residues in most favored region [23] less similarity between query and template may account for it and additionally other statistical parameters are found in support of structure modeled. Plot statistics is collected in figure-3. Glycine and Proline found in regions of acceptance and shown in figure-4.

Plot statistics

Residues in most favoured regions [A,B,L]	298	83.2%
Residues in additional allowed regions [a,b,l,p]	49	13.7%
Residues in generously allowed regions [~a,~b,~l,~p]	7	2.0%
Residues in disallowed regions	4	1.1%
Number of non-glycine and non-proline residues	358	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	39	
Number of proline residues	14	
Total number of residues	413	

Fig. 3- Ramachandran plot statistics of 3-oxoacyl-acyl-carrier protein synthase I/II

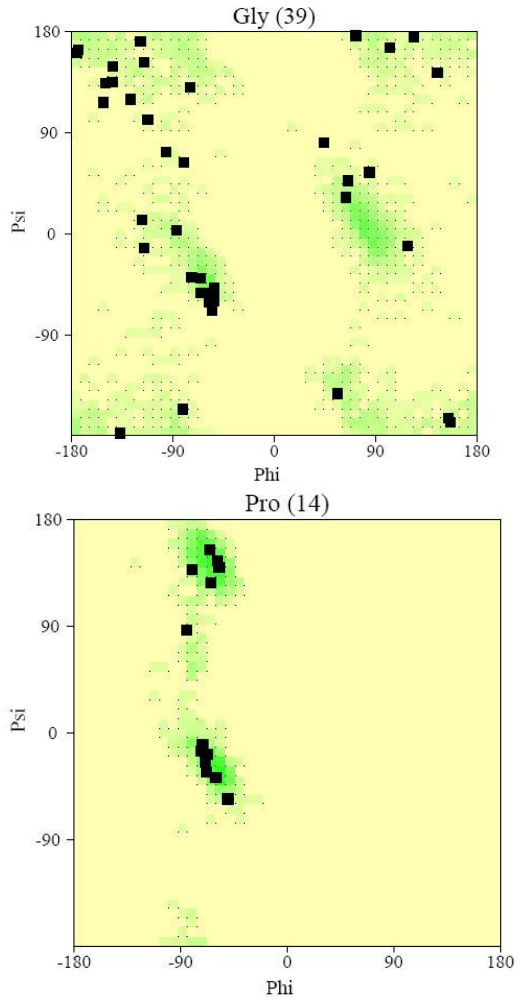

Fig. 4- Ramachandran plot for Glycine and Proline

Main-chain parameters and side-chain parameters calculated at 2.0 Ang. of resolution signifies modeling of 3-oxoacyl-acyl-carrier protein synthase I/II. Root Mean Square Distances from planarity is found below 0.02 when plotted against amino acids frequency in sequence.

Results and Discussion

Development of drug resistance in *Plasmodium falciparum* can be better augmented when structure is available from the present strain. Fatty acid synthesis is discerned as target to inhibit infection and profusion of this parasite. Enzyme structure modeling undertaken in present work could be a tool to study better structural characteristics of 3-oxoacyl-acyl-carrier protein synthase I/II for drug design community. Functional part from structure after recognition can be set in structure based drug design codes. Modeling studies manifested good stereochemical placement of main chain parameters. Bond angles and bond lengths are under confined limits although side chain modeling introduced some levels of displacement of residues beyond most favored regions. Catalytic site in enzyme structure can be examined after screening catalytic databases available. More efforts in structural analysis in concern with mutational studies can provide better insight towards development of drug resistance profiles of this parasite. Thus present studies of modeling of 3-oxoacyl-acyl-carrier protein synthase I/II has brought future prospective to fight against malarial infection and provide better health standards for community.

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