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Comprehensive structural analysis of the open and closed conformations of *Theileria annulata* enolase by molecular modelling and docking

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ABSTRACT

Theileria annulata is an apicomplexan parasite which is responsible for tropical theileriosis in cattle. Due to resistance of *T. annulata* against commonly used antitheilerial drug, new drug candidates should be identified urgently. Enolase might be a druggable protein candidate which has an important role in glycolysis, and could also be related to several cellular functions as a moonlight protein. In this study; we have described three-dimensional models of open and closed conformations of *T. annulata* enolase by homology modeling method for the first time with the comprehensive domain, active site and docking analyses. Our results show that the enolase has similar folding patterns within enolase superfamily with conserved catalytic loops and active site residues. We have described specific insertions, possible plasminogen binding sites, electrostatic potential surfaces and positively charged pockets as druggable regions in *T. annulata* enolase.

1. Introduction

Enolase is mainly responsible for catalyzing the interconversion of 2-phosphoglycerate (2-PGA) and phosphoenol pyruvate (PEP) in glycolytic pathway. The enzyme has also several pathogenic features such as plasminogen and fibronectin binding activities (Avilán et al., 2011; Bao et al., 2014; Toledo et al., 2012). Despite the non-glycolytic functions of enolase has not been clarified completely yet (Paludo et al., 2015), the enzyme has sophisticated roles such as being part of protein clusters related to transcription, development, growth, aging, death, apoptosis in cells and they have been named as "moonlighting" functions (Avilán et al., 2011; Paludo et al., 2015).

Tropical theileriosis is caused by the apicomplexan parasite *Theileria annulata* which is transmitted by a tick vector from the genus *Hyalomma* in cattle (Echebli et al., 2014; Li et al., 2014). *Theileria* parasites invade the leukocytes by sporozoites secreted from the vector, schizonts mature into merozoites and infect erythrocytes subsequently; leading to high rate of morbidity and mortality (Mans et al., 2015; Razavi et al., 2011; Sharifiyazdi et al., 2012). Some recent studies reported that *T. annulata* has developed resistance to buparvaquone; a well-known drug used in the treatment of theileriosis (Marsolier et al., 2015; Mhadhbi et al., 2010; Sharifiyazdi et al., 2012). Therefore; *T. annulata* enolase could be a possible target for new drug-design studies because of emerging requirement for alternative drugs against the parasite.

Homology modeling is one of the prominent step in structure-based drug design studies and provides information to estimate 3D structure and druggable candidate sites of molecular targets in the absence of experimentally solved 3D structures (Agrawal, 2013). The modeled structures ensure information about functional and evolutionary features of the target proteins (Wallner and Elofsson, 2005). Furthermore, molecular docking is used to determine optimum binding modes of ligands to a certain site of protein target in structure-based drug design (Thomsen and Christensen, 2006). Present study has been conducted to evaluate druggable potential of *Ta*ENO for new drug design studies. The 3D open and

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closed conformation models of *Ta*ENO were built by homology modeling approaches following the analysis of the enzyme by amino acid sequence alignment. Primary and secondary structure analysis, the active site determination and docking of the substrate (2-PGA) were also performed.

2. Materials and methods

2.1. Sequence alignment and primary structure analysis

Enolase amino acid sequences from *Theileria annulata* (Accession number: ADU859739), *Theileria parva* (Accession number: XP_764336), *Toxoplasma gondii* (Accession number: 3OTR_A GI:307568483), *Plasmodium falciparum* (Accession number: AAA18634), *Lycopersicum esculentum* (Accession number: X58108), *Bos taurus* (Accession number: AAI02989 is muscle enolase), *Homo sapiens* (Accession number: 3UCC_A GI:401871303 is muscle enolase), *Saccharomyces cerevisiae* (Accession number: P00924) were obtained from NCBI protein database and aligned by using MUSCLE multiple sequence alignment tool from The European Bioinformatics Institute (EMBL-EBI) (http://www.ebi.ac.uk/Tools/msa/muscle) (Edgar, 2004) to perform multiple protein sequence analysis. Physiochemical properties of *T. annulata* enolase were estimated by ProtParam tool from ExPASy portal (http://web.expasy.org/protparam) (Gasteiger et al., 2005).

2.2. Secondary structure and domain analysis

Secondary structure elements (alpha helices, beta strands and random coils) of the *T. annulata* enolase were identified by using PSIPRED protein sequence analysis workbench (http://bioinf.cs.ucl.ac.uk/psipred) (Buchan et al., 2013). PSIPRED ver3.3 server was selected to predict secondary structure because of high accuracy of the prediction criteria which is based on position specific scoring matrices created by PSI-BLAST (Position-Specific Iterated BLAST) search (Jones, 1999). Domain analysis was done using CATH-Gene3D protein structure classification database (Class, Architecture, Topology, Homology) by FASTA sequence search option (Sillitoe et al., 2015).

2.3. Template selection and homology model building

Enolase possesses “open”, “closed” and “incompletely closed” active-site structures (Avilán et al., 2011). In this study; open and closed conformation models of *T. annulata* enolase were built and compared with each other. The first step in homology model generation is to find a suitable template structure which is solved by experimental methods such as NMR and X-ray crystallography (Cavasotto and Phatak, 2009; Krieger et al., 2003). Template structures were identified by using NCBI/BLAST server (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and PSI-BLAST search (BLOSUM62 matrix, Expect threshold, 10) was carried out against the PDB (Protein Data Bank) proteins by using FASTA sequence format of *T. annulata* enolase (Accession no: ADU859739). Template structures were selected based on Max score, Query cover, E-value and identity percentage. Atomic coordinates of the template proteins (PDB file format) were retrieved from the RSCB Protein Data Bank (www.rcsb.org).

Three-dimensional model of the open conformation of *Ta*ENO was generated by the method of homology modeling using MODELLER ver9.12 software (Eswar et al., 2006) which uses template structure file and target amino acid sequence to build models. Selection criteria of the best model based on the Discrete Optimized Protein Energy (DOPE) and normalized DOPE (z-DOPE) scores among 50 models. The closed conformation model of *Ta*ENO was built using MODELLER ver9.15 software (Eswar et al., 2006) and the best model was selected among 100 models, based on the scores mentioned above.

Energy minimization therewithal geometry of the selected models were optimized by applied AMBER force field 14SB and using Steepest Descent algorithm from UCSF Chimera ver1.10.1 software (Pettersen et al., 2004). PyMOL software was used to superimpose selected templates and model proteins, and to calculate the RMSD (Root-Mean-Square Deviation) value according to the C-alpha traces (The PyMOL Molecular Graphics System, ver1.7.4 Schrödinger, LLC). Domains of *Ta*ENO were visualized by Molsoft ICM-Browser (MolSoft LLC, La Jolla, CA, USA). Poisson-Boltzmann electrostatics calculations of open and closed conformations were carried out by PDB2PQR server ver2.0.0 (Dolinsky et al., 2004). At the beginning, PROPKA ver3.0 was used to assess protonation states at pH 7.4, applying AMBER99 force field provided from the server (Olsson et al., 2011) and generate APBS input files. Then APBS ver1.3 (Adaptive Poisson-Boltzmann Solver) was launched to calculate Poisson-Boltzmann Electrostatics (PBE) (Baker et al., 2001). Electrostatic potential coloring was visualized, total solvent excluded (SESA) and accessible (SASA) surface areas were calculated by Chimera ver1.10.2 software (Pettersen et al., 2004).

2.4. Protein model validation

Generated protein models were subjected to various types of validation steps. ERRAT ver2.0 server was used to analyze statistics of non-bonded interactions between atoms (Colovos and Yeates, 1993). Ramachandran plot of the model proteins were carried out by using RAMPAGE server (Lovell et al., 2003). Protein quality was assessed by ProQ and ProSA servers which recognizes errors in 3D structure of protein models and give over-all protein quality score, knowledge-based energy graph and LGscore respectively (Wallner and Elofsson, 2003; Wiederstein and Sippl, 2007).

2.5. Active site prediction and docking of 2-phosphoglyceric acid (2-PGA)

Druggability assessment and potential active site pockets of *T. annulata* enolase was predicted by DoGSiteScorer; Active Site Prediction and Analysis Server (Volkamer et al., 2012) and 3DligandSite; Ligand-binding site prediction server (Wass et al., 2010). Structure of 2-phosphoglyceric acid was retrieved from the NCBI PubChem Database (http://pubchem.ncbi.nlm.nih.gov) with the accession number of 59 as SDF file format. Conformer with the lowest energy was predicted by MarvinSketchver15.2.9, 2015, ChemAxon (http://www.chemaxon.com) and used in docking studies. Binding modes of the 2-PGA to the active site pocket of *Ta*ENO were predicted by both of AutoDockVina and Molegro Virtual Docker softwares (Thomsen and Christensen, 2006; Trott and Olson, 2010). Programs predict binding modes of the substrate and calculate the energy of particular pose, based on the scoring function which is described by the summation of intra- and intermolecular forces (Thomsen and Christensen, 2006; Trott and Olson, 2010). Binding modes of 2-PGA in protein model were visualized by PyMOL (The PyMOL Molecular Graphics System, Ver1.7.4 Schrödinger, LLC).

3. Results

3.1. Sequence analysis

Catalytic residues, catalytic flexible loops, possible insertions and plasminogen binding sites of *T. annulata* enolase were evaluated by sequence alignment, using the information available in the literature (Akat et al., 2014; Bergmann et al., 2003;