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Computational 3D Structure Prediction, Evaluation and Analysis of Pyruvate Dehydrogenase an Effective Target for Filarial Infection by *Brugia pahangi* Using Homology Modeling Approach

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ABSTRACT
Pyruvate dehydrogenase protein is a part of cellular respiration which helps in maintaining glucose and energy level (ATP) in the cells for their normal functioning. Initially it was reported that *Brugia pahangi* is a nematode causing filarial infections in animals like dogs and cats. But, in the recent studies, *Brugia pahangi* is also found to cause filarial infections in Humans, in Kuala Lumpur, Malaysia. By targeting the cellular respiration of this worm this filarial infections can be treated. The three dimensional structure of pyruvate dehydrogenase in *B. pahangi* will help to target cellular respiration more efficiently. The comparative modeling approach based software Modeller9.13 is used to model three dimensional structure of pyruvate dehydrogenase. The modeled structure was evaluated by Procheck, VADAR, Process, ProSA tools. The Physiochemical properties of this protein are analyzed by APD2 and Protparam software. Enzymatic Cleavage site of this protein is identified by Peptide cutter tool. Active sites in modeled structure are identified by Pocket finder tool for drug targets. The modeled structure, properties and active sites of this protein will help to design lead molecules which can treat the filarial infections in humans. The structure will also help to understand the working of pyruvate dehydrogenase in better and efficient manner by identifying the protein interaction networks in *Brugia pahangi*.

Keywords: Filarial, Comparative modelling, Active site, Procheck, Cleavage Site, interaction networks.

INTRODUCTION

Brugia pahangi commonly named as filarial nematode worm belongs to animalia kingdom, nematode phylum, secernentea class, spirurida order, onchocercidae family and brugia genus. The taxonomy of the worm is available at http://www.uniprot.org/taxonomy/6280. The pyruvate dehydrogenase complex (PDC) activity is very important to maintain blood glucose and ATP levels, which largely depends on the phosphorylation status by pyruvate dehydrogenase kinase (PDK) isoenzymes. This protein helps in transforming pyruvate to acetyl-coA, via Pyruvate decarboxylation. These connections make it a very important connecting link between glycolysis and citric acid cycle. Although it has been reported that PDC is phosphorylated and inactivated by PDK2 and PDK4 in metabolically active tissues including liver, skeletal muscle, heart, and kidney during starvation and diabetes, the precise mechanisms by which expression of PDK2 and PDK4 are transcriptionally regulated still remains unclear. [1]

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Filarial nematode causes a number of serious diseases in humans (e.g., lymphatic filariasis and onchocerciasis) and animals (e.g., canine heart worm disease). Filarial parasites have complex life cycles that share the following general features: Arthropod vectors (ticks, mites, mosquitoes, black flies) ingest microfilariae (Mf) that circulate in the blood or live in the skin of definitive vertebrate hosts. [2] Recently in 2011, at suburban area of Malaysia *B. pahangi* based filarial infection was observed in Human. This is very rare case of filarial infection by *B. pahangi.* [3] A relatively low level of isocitrate dehydrogenase is present in the mitochondria of *B. pahangi*. It acts as a homolactete fermenter under *in-vitro* conditions. They don't require oxygen for either survival or motility. They possess highly cristated mitochondria. The activities of some of the tricarboxylic acid cycle enzymes of adult *B. pahangi* also were assayed to examine other possible reasons for the lack of an oxygen dependent energy metabolism in this helminthes. *Brugia pahangi* was obtained from the peritoneal cavity of infected gerbils. They have a high endogenous rate of lactate formation, presumably from the dissimilation of glycogen. *B. pahangi* metabolizes stored carbonhydrate rapidly and if the worms are incubated in glucose, the production of lactate which accumulates arises from endogenous as well as exogenous pools. [4] This protein

is very important in metabolic pathways of *B. pahangi* and hence, by blocking this target protein prevention from filarial infection can be achieved. Here three dimensional structures modelling of pyruvate dehydrogenase is carried out by homology modelling based approach. This three dimensional structure can be further used for drug designing and interaction studies in metabolic pathways of *Brugia pahangi.*

MATERIALS AND METHODS

Model Building

The linear amino acid sequence of pyruvate dehydrogenase from *Brugia pahangi* was retrieved from protein sequence database of NCBI (http://www.ncbi.nlm.nih.gov/). The Accession No: ABO84944, Version: ABO84944.1, GI: 140084473 was taken as target protein sequence if 115 aminoacids. Here we have followed Advanced modeling methodology of the MODELLER9.13 to produce a better structure. [5] The modeling of 3D structure of the target proteins followed a sequential order, starting from templates selection from PDB (protein data bank) related to the target sequence using BLASTP (protein-protein alignment). There were number of hits from PDB were obtained when sequences are compared, potential structures (PDB-ID: 1NI4, 1OLX, 1W85, 2OZL, 3DUF, 3EXE), were taken for model building. The templates were first aligned with each other using compare.py script available in homology protein modeling program of MODELLER9.13. Using this alignment the templates and target were aligned using align2d.py script. On the basis of this alignment, ten comparative models of the target (pyruvate dehydrogenase) were generated by MODELLER9.13, applying the build_model.py script. In the first step of model building, distance, bond length, bond angle, H bond potential and dihedral angle restraints on the target sequence were derived from its alignment with the template 3D-structure.

Evaluation of model

The best model was selected by picking the model with lowest energy value of the Modeller objective function, dope score and highest value of molpdf score from a collection of models generated by MODELLER. Modeller objective function, molpdf and DOPE score are the statistical parameter for assessment of model using the standard Modeller Energy function. The sixth model generated had the highest molpdf score (3282.21875) thus selected as the model containing 3D structure of the target protein. We are not going for loop refinement because the structure generated is continuous without any gap or unassigned coordinates. The model selected was then passed through the process of energy minimization. The process of energy minimization provides new coordinates to the input structure to make it energetically sounder. The new coordinates are having fewer differences to the structure generated by MODELLER. This process was implemented to make the predicted structure closer to the form in which this protein occurs in nature. For this purpose we used SPDBV software in which, after minimizing the structure energetically for 30 iterations the coordinates were accepted as the variation in the energy became very small between two iterations.

The Stereochemical qualities of the models are checked by PROCHECK (http://www.ruppweb.org/ftproot/Procheck_NT.zip) [6] and ProSA (https://prosa.services.came.sbg.ac.at/prosa.php). [7]

Model Analysis

It is very much necessary to analyze the structure modeled; this provides statistical reliability to the structure. For analyzing structure we used a number of tools. VADAR is an online tool that predicts the reliability of a protein. On submission of our modeled protein it provided us with different analysis. The red, yellow and green regions represent the favored, allowed, and "generously allowed" regions as defined by VADAR. [8]

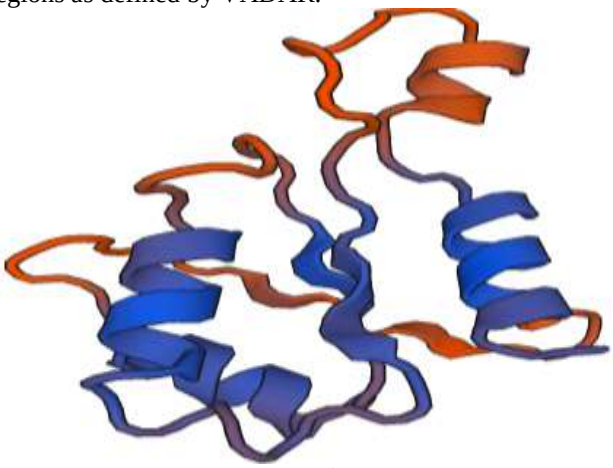

Fig. 1: Three dimensional structure of the modeled protein in Molecular Viewer

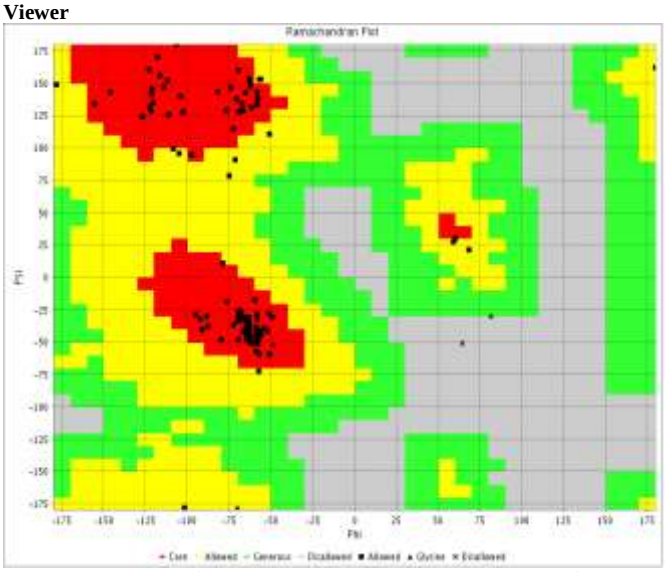

Fig. 2: Calculation of stability through Ramachandran plot of modeled protein using VADAR server. (http://vadar.wishartlab.com/). Red region is core and yellow is called allowed region

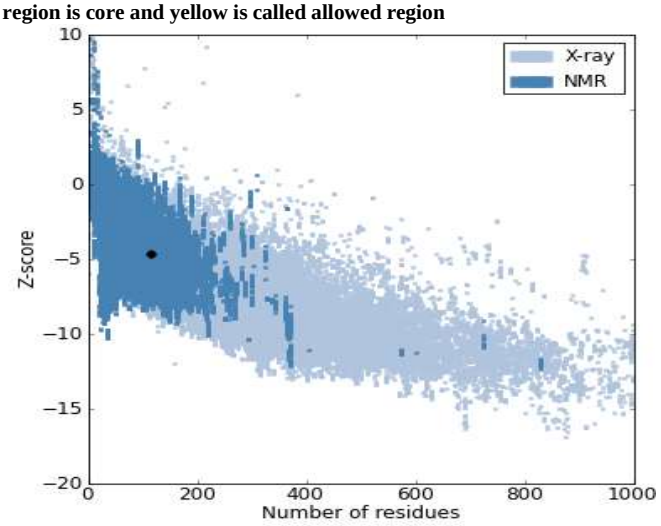

Fig. 3: ProSA Results of modeled pyruvate dehydrogenase showing point in range of NMR and X ray structure

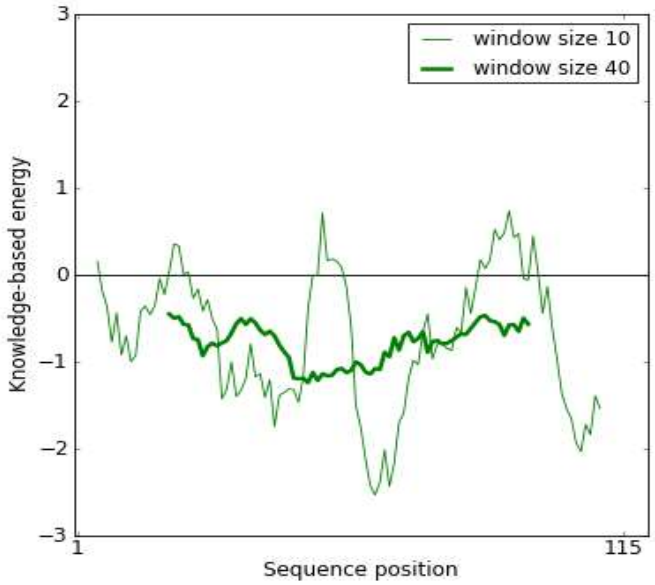

Fig. 4: Energy of modeled structure in fraction of window size of 10 (light green) and 40 Amino acids (dark green)

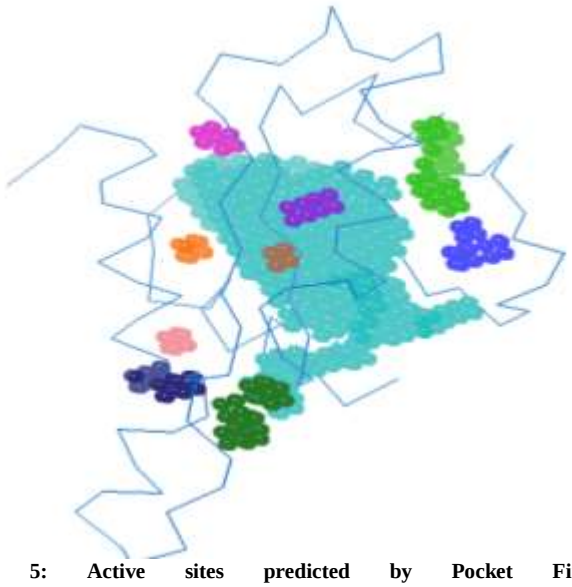

Fig. 5: Active sites predicted by Pocket Finder (http://www.modelling.leeds.ac.uk/cgi-bin/pocketfinder/pfmage.cgi) contain 10 active sites cyan color is largest site in the protein

According to ProsA server analysis the Z-score of the predicted protein is -4.63. The *z*-score indicates overall model quality (Fig. 3). Its value is displayed in a plot that contains the *z*-scores of all experimentally determined protein chains in current PDB. In this plot, groups of structures from different sources (X-ray, NMR) are distinguished by different colors (light blue and dark blue respectively). It can be used to check whether the *z*-score of the input structure is within the range of scores typically found for native proteins of similar size. The second plot shows local model quality by plotting energies as a function of amino acid sequence position *i*. In general, positive values correspond to problematic or erroneous parts of the input structure. A plot of single residue energies usually contains large fluctuations and is of limited value for model evaluation. Hence the plot is smoothed by calculating the average energy over each 40-residue fragment *s (i, i+39)*, which is then assigned to the 'central' residue of the fragment at position *i+19*. A second line with a smaller window size of 10 residues is shown in the background of the plot (Fig. 4). [7]

Protein properties and Active site analysis

According to APD2 (http://aps.unmc.edu/AP/prediction/prediction_main.php) (Antimicrobial Peptide Predictor), the molecular weight is 12582.694, total hydrophobic ratio is 42% and Protein-binding Potential (Boman index) is: 1.28 kcal/mol. [9] The estimated half-life is: 100 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (*Escherichia coli*, *in-vivo*). The expected PI is 6.99 as predicted using Protparam (http://web.expasy.org/protparam/). The enzymes that can cleave this protein are Arg-C proteinase, Asp-N endopeptidase + N-termial Glu, BNPS-Skatole, CNBr, Chymotrypsin-high specificity (C-term to [FYW], not before P), Chymotrypsin-low specificity (C-term to [FYWML], not before P), Clostripain, Formic acid, Glutamyl endopeptidase, Iodosobenzoic acid, LysC, LysN, NTCB (2-nitro-5-thiocyanobenzoic acid), Pepsin (pH1.3), Pepsin (pH>2), Proline-endopeptidase, Proteinase K, Staphylococcal peptidase I, Thermolysin, Trypsin according to peptide cutter software (http://web.expasy.org/peptide_cutter/) by EXPASY. [10] Pocket-Finder works by scanning a probe radius 1.6 angstoms along all gridlines of grid resolution 0.9 angstroms surrounding the protein. The probe also scans cubic diagonals. [11-13]

According to InterProSurf Protein-Protein Interaction Server (Probe radius: 1.400, POLAR area/energy = 2673.65, APOLAR area/energy = 4917.60, Total area/energy = 7591.25, Number of surface atoms = 602 and Number of buried atoms = 273). InterProSurf is designed to predict the most likely sites on proteins to interact with other proteins, such as toxin elements, cell receptors and other proteins that make up virus capsids. [14]

RESULT

The structure of linear amino acid sequence of pyruvate dehydrogenase subunit *Brugia pahangi* (Accession No: ABO84944, Version: ABO84944.1, GI: 140084473) was modeled by Modeller Software. The structure contains 6 helix and 2 sheet regions in the entire structure. The result of Pocket Finder says that this protein contains 10 active sites. According to VADAR analysis the structure is very much stable and closer to the form it might be in nature. The Ramachandran plot generated by VADAR says that one two amino acid is in the disallowed region and rest all are closer to the core region of this plot. The ProSA server result predicted that the predicted structure is having its z-score -4.63, which is in the range of the z-scores of the currently available structures from NMR various enzymatic cleavage sites are predicted in protein.

DISCUSSION

The modeled structure is verified on various evaluation criteria and it signifies that the structure modeled is very much perfect and follows all the criteria protein determined by experimental method. This model can be further used to design drug and other structure based analysis.

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