

HOMOLOGY MODELING OF SUBCUTANEOUS FILARIASIS DHFR PROTEINS

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ABSTRACT

Objective: A systematic technique for protein modeling offers great assistance in the study of protein function, dynamics, interactions with ligands, and other proteins and even in drug discovery and drug design. Subcutaneous filariasis is rare parasitic disease caused by *Loa loa* (eye worm) and *monsonallastreptoscerca species*.

Methods: The present study develop three dimensional structure of dihydrofolatereductase present in *Loa loa* species. For this purpose knowledge based homology modeling is used by using Schrodinger Glide 5.6 software.

Results: The procedure involves alignment that maps residues in the query sequence to residues in the template sequence to generate structural model of target, which was further refined and final result validated by using Ramchandran plot.

Conclusion: In ramchandran plot majority of the amino acids are in the phi-psi distribution and the develop model is reliable and of good quality.

Keywords: Subcutaneous filariasis, Loa Loa, Homology modeling

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INTRODUCTION

Dihydro-folate-reductase (DHFR) is a key enzyme in folate metabolism; it plays a major role in the biosynthesis of nucleic acid through the catalysis of the NADPH reduction of 7, 8-dihydrofolate to 5, 6, 7, 8-tetrahydrofolate. DHFR protein is main target in subcutaneous filariasis. This inhibitor also have anticancer, antibacterial and even antimalarial activity, which is collectively known as anti-folates. *Loa loa* is a thread like worm that lives under the skin in subcutaneous fat causing loiasis [1]. It is one of three parasitic filarial nematodes that cause loiasis in human. The other two are *monsonallastreptoscerca* and *onchocercavolvulus* [2]. Homology modeling also known as comparative modeling of protein is the technique which allows to construct an unknown atomic-resolution model of target protein from its amino acid sequence and an 3D structure of a related homologous protein (the template) [3]. Therefore the present study focused on to build a high quality of protein 3D structure for subcutaneous filariasis DHFR protein.

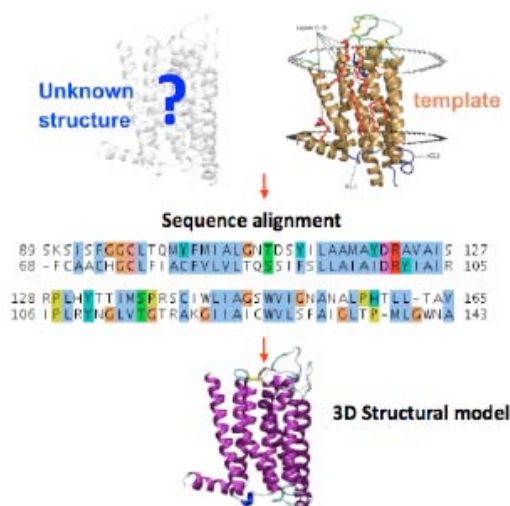
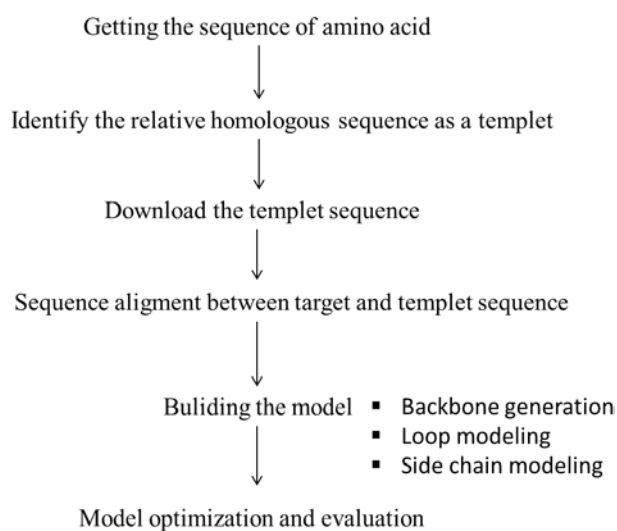


Fig. 1: Homology modeling

Homology modeling

The protocol to the model building by homology modeling (scheme 1) involves template identification, alignment of the sequences, generation of the model coordinates, optimization, and validation of the model [4]. The process start with getting the amino acid sequence then identify related homologous sequence as template. In next step download the template sequence, the sequence alignment between target and selected template, then build up the model and optimize and evaluate (fig. 1) [5-6].



Scheme 1: Protocol for homology modeling

MATERIALS AND METHODS

1) Amino acid sequence and identify related homologous sequence: Amino acid sequence of DHFR protein for subcutaneous filariasis was downloaded from www.unipro.org. And DHFR malaria select as

template which is homologous to target protein. Template sequence also download from same website.

2) Sequence alignment: A search with BLAST against this database will give a list of known protein structures that match the sequence. BLAST will provide a list of possible templates for the unknown structure. In sequence alignment align the target sequence and template sequence.

3) Building the model: In this step factor involve to build the model is: 1) backbone generation 2) loop modeling 3) side chain modeling. There are two type of model: knowledge base model and energy based model. By using knowledge based model subcutaneous filariais protein

was build up. Further efficiency was improve by using refining loops. There are three refinement task available in refine structure stepi. e refine loops, predict side chain and minimization.

4) Evaluation: The model was further evaluated by using ramachandran plot.

RESULTS AND DISCUSSION

1) Basic local alignment search tool: One of the most widely used algorithms for comparing primary biological sequences, such as aminoacid sequences, is BLAST (Basic Local Alignment Search Tool). In this search the DHFRloaloa amino acid sequence compare with DHFR malaria sequence shown in fig. 2.

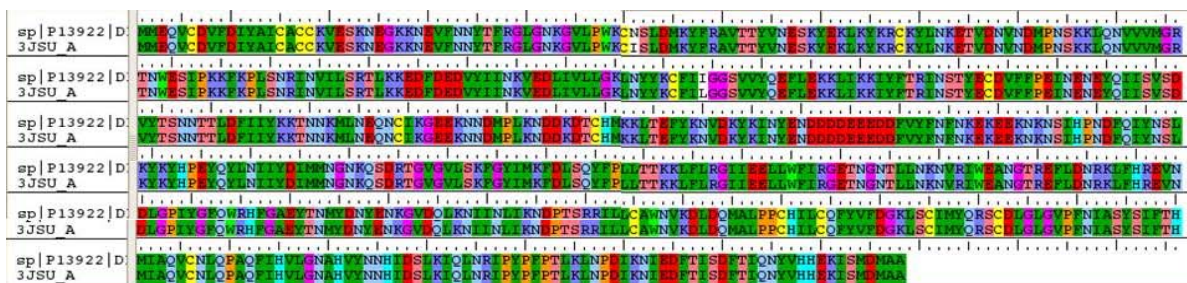


Fig. 2: Basic local alignment search tool

2) Template selection: After BLAST, result as a list of 3D protein structures ordered according to their "sequence identity percentage" with the target sequence. The quality of the homology model is

dependent on the quality of the sequence alignment and template structure. Model quality declines with decreasing sequence identity. Highest percentage identity template was selected shown in fig. 3.

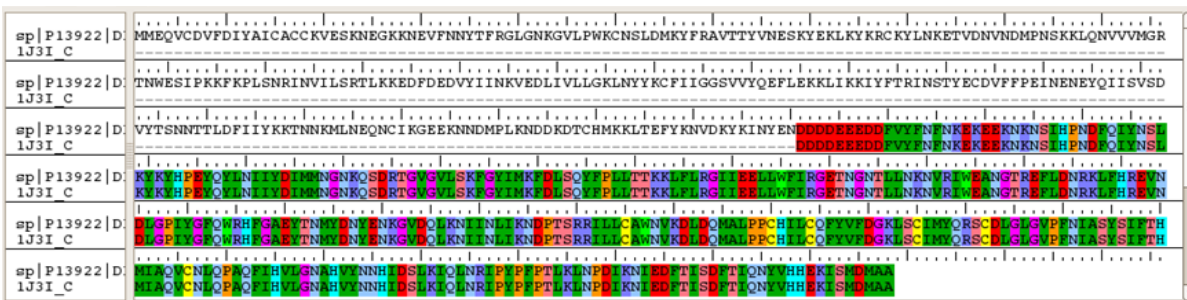


Fig. 3: Template selection

3) Sequence Alignment: For model development, sequence alignment file between DHFR loaloand the selected DHFR malaria template sequence was carried out shown in fig. 4.

The structure produced in the Build Structure step is likely to represent only a local energy minimum and not the global minimum.

4) Building model: The Build Structure program builds insertions, closes gaps, and predicts side-chain conformations.

Therefore, regions with gaps in the alignment are likely to require refinement in the Refine Structure step shown in fig. 5.

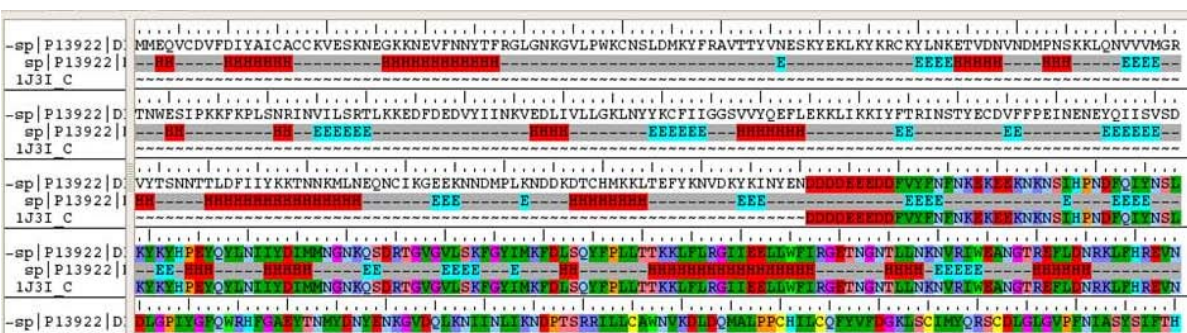


Fig. 4: Sequence alignment

-sp P13922 D: sp P13922 I: 1J3I_C	MMEQVCDVFDIYAICACCKVSKNEGKKNEVFNNTFRGLGKNGVLPWKCNSLDMKYFRAVTTYVNESKYKLYKRCYKLNKETVDNVNDMPNSKCLKQNVVVMGR
-sp P13922 D: sp P13922 I: 1J3I_C	TNWESEPCKFKPLSNRINVLRSRTLKKEDFDEDVYIINKVEDLIVLLGKLNYYKCFIIGGSVYVQEFLEKLIKKIYFTRINSTYECDVFPPEINENEYQIIISVSD
-sp P13922 D: sp P13922 I: 1J3I_C	VYTSNNTTLDFLIYKKTNNKMLNEQNCIKGEEKNNDMPLKNDKDKTCHMKKLTPEYKRVDKYKINYENDDDEEDDFVYFNFNKEKEEKNNKSIHPNDFQIYNLSL
-sp P13922 D: sp P13922 I: 1J3I_C	KYKYHPEYQYLNIYDIMMNGNKQSDRTGCVGLSKFGYIMKFDLSQYFPLLTTKKLFLRGIIEELLWFI RGETNGNTLLNKVRIWEANGTREFLDNRKLFHREVN
-sp P13922 D: sp P13922 I: 1J3I_C	DLGPIYGFQWRHFGEAYTNNYDNYENKGVQLKNIINLIKNDPSTRRIILCAWNVKDLQMALPPCHIILCQFYVFDGKLSCIMYQRCSDLGLGVPFNIAYSIFTH

Fig. 5: Building model

5) Refine model: To improve the structure most efficiently the refinement of model required. There are three refinement tasks was carried out in the Refine Structure step: Refine loops, Predict side chains, and minimize.

6) Model analysis: Minimize model was further evaluated by using ramachandran plot. The Ramachandran plot for the generated model and the template protein (DHFR malaria) is shown in fig. 6.2b. From the plot, it is revealed that majority of the amino acids are in the phi-psi distribution and the model is reliable and of good quality.



Fig. 6.1: a) Homology modelled structure of DHFR subcutaneous filariasis

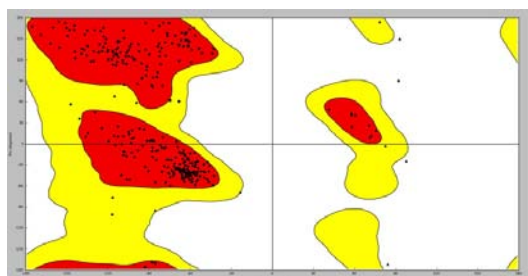


Fig. 6.2: b) Ramachandran plot of modelled structure of subcutaneous filariasis

CONCLUSION

The present study was carried out to examine the probable role of DHFR protein in subcutaneous filariasis. No X-ray crystal structure of DHFR subcutaneous filariasis was available at PDB. Hence, homology modelling approach was used to obtain the 3D-structure of DHFR subcutaneous filariasis protein using the structure of DHFR malaria as a template. The modelled structure was subjected to energy minimization and refinement. Refine structure was validated by using ramachandran plot. In ramachandran plot majority of the amino acids are in the phi-psi distribution and the develop model is reliable and of good quality.

CONFLICT OF INTERESTS

Declared none

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