Homology modeling of fatty acid and retinoid binding (FAR) protein from the filarial nematode *Acanthocheilonema viteae*

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**ABSTRACT**

Two structurally novel classes of small helix-rich Fatty acid and retinoid binding (FAR) proteins are produced by parasitic nematodes. The plant and animal hosts of these parasitic nematodes have no counterparts of these proteins. FAR proteins play an active role in nematode development and survival when in contact with host tissue. Thus FAR proteins represent potential targets for new synthetic drug designing. A 3-dimensional model (3D) was designed for the Fatty acid and retinoid binding protein (178 aa) from the filarial nematode *Acanthocheilonema viteae*. A predicted model was constructed for the target protein using SWISS-MODEL. The constructed model was validated using protein structure checking tools for reliability. The prediction of 3D structure of a protein is a prerequisite and is also required for identifying the conformational epitopes which are essential for synthetic drug designing vaccines. Altogether, these data makes FAR protein a potentially valuable target for drug and vaccine development against filarial nematodes.

**Key words:** 3D structure, *Acanthocheilonema viteae*, Drug, FAR protein, Filarial nematode, SWISS-MODEL

**INTRODUCTION**

Parasitic nematodes are creating a major global ecological, economical and medical threat not only to plants and animals, but also towards human beings. To date, approximately 30,000 nematode species have been identified1, however, the total estimated number of species is over one million2. Worldwide among the known species of nematodes, more than 16,000 species are parasites of plants, animals and humans, causing various diseases of socio-economic importance3,4. Across the world about 160 million people are affected by the filarial nematodes, which are the causative agents of tropical diseases, filariases5. *Acanthocheilonema viteae*, previously known as *Dipetalonema viteae*, is a rodent filarial worm that is often used as a model to study human filarial infections. Adult filarial nematodes are usually found in the body cavities and/or subcutaneous tissues of their mammalian host6. *Acanthocheilonema viteae* shares considerable antigenic homology with the human filarial worm *Onchocerca volvulus*7.

FARs promote the absorption, transportation and specific localization of fatty acid and retinoid8,9. Retinol plays important roles in gene activation, cell signaling, and tissue differentiation and repairation5,10,11. Thus the secreted FARs can help nematodes not only to obtain the lipid nutrition from the host, but also to infect the host and inhibit the host defense mechanism12.

Nematodes require fatty acids and retinol for lipid biosynthesis and assembly of macromolecular structures, including the cuticle and developing embryos13,14. Parasitic nematodes are unable to synthesize fatty acids and retinol de novo in order to satisfy various biological requirements15. While free living stages presumably acquire fatty acids from the environment, parasitic stages must have access to host-derived stores. This makes the characterization of novel fatty acid binding proteins important for understanding both nematode biology and pathogenesis. The fatty acid and retinoid binding (FAR) proteins make up the second class of nematode fatty acid binding proteins. FAR proteins are single domain proteins of approximately 20 kDa that exhibit a unique α-helix rich coiled coil structure compared with mammalian fatty acid binding proteins10.

Currently, neither efficient drugs nor vaccines are available to eliminate or prevent filarial infections, hence 3D structure prediction of FAR protein provides the new target area for development of control strategies against filarial infections.
MATERIALS AND METHODS

Retrieval of target sequence:
The amino acid sequence of the Fatty acid and retinoid binding protein of the filarial nematode Acanthocheilonema viteae was obtained from the sequence database of NCBI http://www.ncbi.nlm.nih.gov/entrez GenBank: AAM28244.1. The protein is 178 amino acids in length. It was ascertained that the three-dimensional structure of the protein was not available in Protein Data Bank, hence the present exercise of developing the 3D model of the Fatty acid and retinoid binding protein of the filarial nematode Acanthocheilonema viteae was undertaken.

Template searching:
An attempt was made to find a suitable template protein for the modeling of the target protein. The template protein was searched through BALST, which is an online tool for searching similar sequences, based on sequence similarity. From the homology searching, one template was selected. Crystallography structure of Lipid Binding Protein CE-FAR-7 from Caenorhabditis elegans [PDB: 2w9yA] was selected as template protein.

Homology modeling and structure refinement:
The secondary structure of the Fatty acid and retinoid binding protein of the filarial nematode Acanthocheilonema viteae was predicted using PDB-SUM tool. A rough 3-D model of FAR protein was constructed from the sequence alignment between and the template proteins using SWISS-MODEL with parameters of energy minimization value. The model was further checked with verify3D and Ramachandran plot at PROCHECK. Accessible surface area prediction using VADAR was performed.

The rough model constructed was solvated and subjected to constraint energy minimization with a harmonic constraint of 100kJ/mol/Å², applied for all protein atoms, using the steepest descent and conjugate gradient technique to eliminate bad contacts between protein atoms and structural water molecules. Computations were carried out in vacuo with the GROMOS96 43B1 parameters set, implementation of SWISS-pdbVIEWER.

Model validation:
The constructed model of Fatty acid and retinoid binding protein of the filarial nematode Acanthocheilonema viteae was examined for validation using different criteria. In the last step of homology modeling the refined structure of the model was subjected to a series of tests for testing its internal consistency and reliability. Backbone conformation was evaluated by the inspection of the Psi/Phi Ramachandran plot obtained from PROCHECK analysis. The Swiss-PdbViewer energy minimization test was applied to check for energy criteria in comparison with the potential of mean force derived from a large set of known protein structures. Packing quality of the refined structure was investigated by the calculation of PROCHECK Quality Control value. The Ramachandran plot of phi/psi distribution in the model is developed using PROCHECK for checking non-GLY residues at the disallowed regions. Standard bond lengths and bond angles of the model were determined. In order to describe the quality of the predicted theoretical model QMEAN4 Z-score was analyzed for describing its absolute quality, as QMEAN4 Z-score is a valuable measure for identifying experimental structures with significant errors.

RESULTS AND DISCUSSION

Structural description of the model:
The functional property of a protein is dependent on its three-dimensional native conformation. Analysis of 3D structure of any protein under study is essential to calculate its interactive nature and its potential role in the biological functions. Detailed study of 3D structures is required for the identification of antigenic determinants and may lead to the designing of new drugs. Computational homology modeling is an established technique which is widely utilized for research and development of drugs. With the help of computational homology modeling the 3D structure of an unknown macromolecule can be predicted by using template of a similar known molecule. The predicted Fatty acid and retinoid binding protein of the filarial nematode Acanthocheilonema viteae has 8 α-helices which are showing 11 helix-helix interactions. The functional native conformation of the predicted protein contains 15 β-turns and 3γ-turns (Figure 1).

Fig.1: Secondary structure of predicted FAR protein of A. viteae
Different protein model analysis programs, including PROCHECK, are explored for the evaluation of the Ramachandran plot quality (Figure 2), and VADAR for the calculation of packing quality.

1. **Ramachandran Plot statistics**

<table>
<thead>
<tr>
<th></th>
<th>No. of residues</th>
<th>%-tage</th>
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<tbody>
<tr>
<td>Most favoured regions</td>
<td>[A,B,L]</td>
<td>111</td>
</tr>
<tr>
<td>Additional allowed regions</td>
<td>[a,b,1,p]</td>
<td>16</td>
</tr>
<tr>
<td>Generously allowed regions</td>
<td>[-a,-b,-1,-p]</td>
<td>1</td>
</tr>
<tr>
<td>Disallowed regions</td>
<td>[xx]</td>
<td>1</td>
</tr>
<tr>
<td>Non-glycine and non-proline residues</td>
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<td>129</td>
</tr>
<tr>
<td>End-residues (excl. Gly and Pro)</td>
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<td>2</td>
</tr>
<tr>
<td>Glycine residues</td>
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<td>1</td>
</tr>
<tr>
<td>Proline residues</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Total number of residues</td>
<td></td>
<td>138</td>
</tr>
</tbody>
</table>

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20.0 a good quality model would be expected to have over 90% in the most favoured regions [A,B,L].

**Fig.2:** Ramachandran Plot of predicted FAR protein of *A. viteae*

The above results are based on the data generated from BLAST analysis, and the X-ray crystallography structure of Lipid Binding Protein CE-FAR-7 from *Caenorhabditis elegans*, used as template protein. SWISS-MODEL was used for generating the predicted model and global energy minimization (Figure 3a). The QMEAN4 Z-scores of the predicted protein is found to be -1.38, which falls in good model category (Figure 3b).

**Fig.3(a):** 3-D structure of predicted FAR protein of *A. viteae* generated by SWISS-MODEL

**Fig.3(b):** QMEAN4 Z-score of predicted FAR protein of *A. viteae*

**CONCLUSION**

Parasitic nematodes are threat not only for the human population but also to the animals and plants of economic importance. At present, there is neither a safe and efficient measure to prevent the nematode infestation nor do to cure it completely. The filarial nematode *Acanthocheilonema vitae* shows considerable similarity in their
antigenic structures with the human filarial nematodes. The secreted FAR proteins are essential for the survival and growth of the parasitic nematodes, as they need them for their life processes. The predicted 3-D structure of FAR protein provides a novel potential synthetic drug target to control these harmful nematodes. The characterization of FAR protein helps in understanding its biochemical nature and how it can be exploited for developing synthetic drugs against parasitic filarial nematodes.

REFERENCES
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