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Full Length Research Paper

Protein modeling of apical membrane antigen-1(AMA-1) of *Plasmodium cynomolgi*

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Apical membrane Antigen-1(AMA-1), an asexual blood stage antigen of *Plasmodium cynomolgi*, is an important candidate for testing as a component of malarial vaccine. The degree of conservation of AMA-1 sequences implies a conserved function for this molecule across different species of *Plasmodium*. Since the AMA-1 of plasmodium cyanomolgi is yet to be structured, the authors have generated a homology model of AMA-1 by using the Swiss-PDB server. The protein's conservity has been verified by performing multiple alignments using Bioedit and conserved domain database. The model was further checked for its correctness by predicting 2D and 3D structures, which validates the structure.

Key words: Protein model, AMA-1, apical membrane antigen-1, *Plasmodium cynomolgi*.

INTRODUCTION

Most of the activities in living organisms are regulated by proteins. All proteins start on a ribosome as a linear sequence of amino acids, this linear sequence must fold during and after the synthesis, so that the protein takes up its native conformation. The native conformation of protein is a stable three dimensional structure that strongly determines a protein's biological function. The knowledge of detailed structural organization is crucial in understanding the role of protein in the cell and the related molecular mechanisms. Insight of three dimensional (3D) structure of a protein are of great assistance when planning experiments aimed at the understanding of protein function and during the drug design process. The experimental elucidation of the 3D structure of the proteins is however often hampered by difficulties to obtain sufficient protein, diffracting crystals and many other technical aspects. The number of solved 3D structures is small, when compared to the rate of sequencing of genes, and no structural information is available for the vast majority of the protein sequences.

At this juncture, prediction methods have gained much interest (Schwede et al. 2003). Genome sequencing projects continuously detect new protein sequences, this provides new information for the application of computational methods, representing a good alternative to the relatively slow experimental processes for predicting protein structure (Rodriguez et al., 1998, West Head et al., 1998).This study focuses on building molecular model of AMA-1 protein of *Plasmodium cynomolgi* using molecular modeling techniques using SWISS MODEL-SERVER (Guex and Peltsch, 1997) as follows: To build a theoretical model for AMA-1 protein by homology modeling and to study the fundamental characteristics of AMA-1 protein by comparative analysis with the parent/template.

The model was generated using SWISS-MODEL v 3.7. an automated protein modeling server available at www.expasy.org.

MATERIALS AND METHOD

Homology/comparative modeling is carried out, if the native sequence similarity with the similar crystal/NMR structure is greater than 35%.The process of building a comparative model is conceptually straight forward. First, an alignment is performed between the sequences for which the structure has been determined by experimental methods (the parent/template) with the sequence to be modeled (the target, in our case AMA-1 of *P. cynomolgi*)

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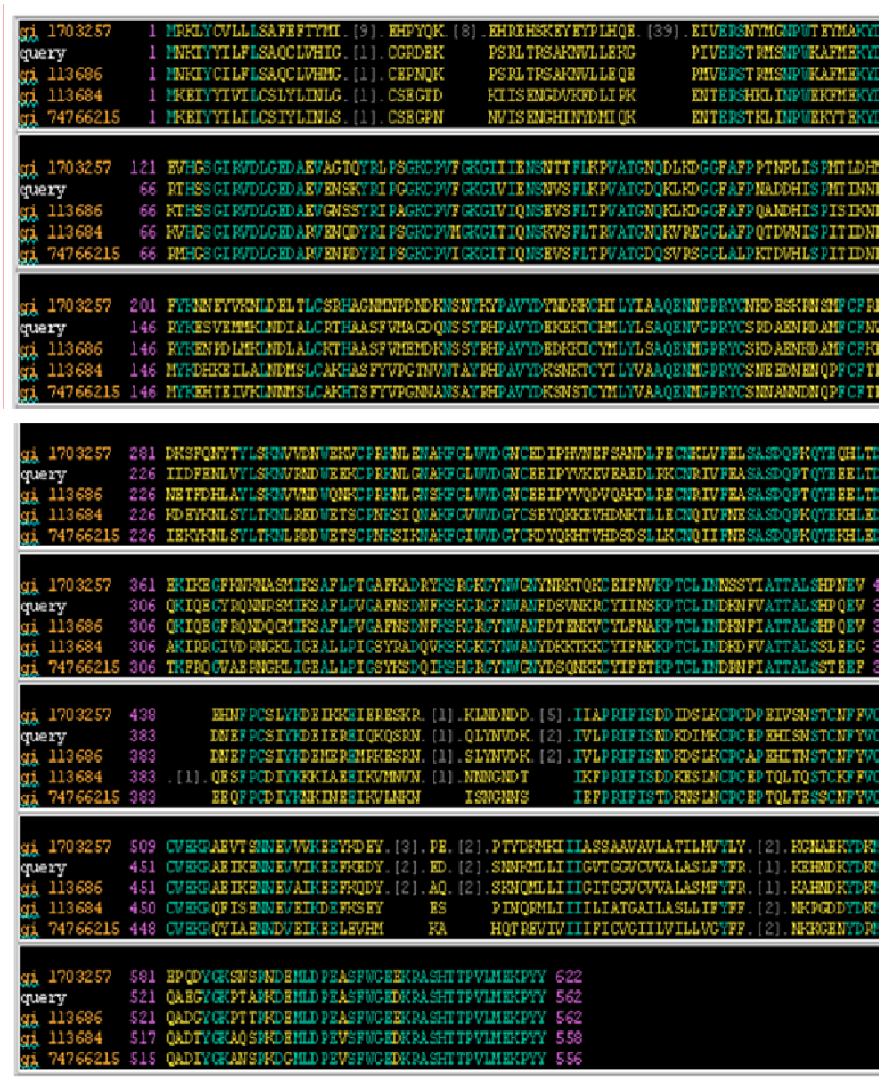

Figure 1. Multiple alignment of AMA-1 protein showing conservity (BIOEDIT).

The comparative model of AMA-1 was built using a web-based homology-modeling server, SWISS-MODEL. It predicts the model in 5 steps. Firstly, it searches for suitable template for the submitted protein to be modeled in the EXNRL-3D database using BLAST P2 program. In the second, step it selects the suitable templates with sequence identity above 25%. Furthermore, this step also detect domains, which can be modeled based on unrelated templates

.Thirdly, jobs are created for PROMOD2 module of SWISS MODEL server, which generates all the probable models of the submitted protein sequence in the fourth step. Fifth step minimizes the energy of all the predicted models with GROMOS96 module of SWISS MODEL server (Van Gunsteren et al., 1996) (GROningen Molecular Simulation). Finally, the best 3D model having minimum energy is sent to the submitter.

The sequence identity of known structure was checked with AMA-1 protein using SIM program that selected all templates with sequence identities above 25%. Furthermore, this step also detected the AMA-1 domains which were further cross checked with CDD (conserved domain database) Garnier et al., (1978). The energy minimization of the generated model was done with GROMOS96 (GROningen, Molecular simulation) (Peitsch, 1995; Peitsch et al., 2000).

Secondary and tertiary structure prediction

The multiple alignments of AMA-1 protein from *Plasmodium* sp. were generated using Bioedit and conserved domain database. The secondary structure of the model was predicted by using NPS@GOR4, predict protein server and SWISS-MODEL server. The model was analyzed for its correctness using PROCHECK.

RESULTS AND DISCUSSION

The multiple alignments of protein sequences (Dutta et.al., 1995) of the AMA-1 of *P. cynomolgi* highlights the degree of sequence similarity (Cheng and Saul, 1994) and high degree of sequence conservation with the templates selected, which implies the conserved function for this molecule across the different species of *Plasmodium* Figure 1,
http://www.ncbi.nlm.nih.gov/BLAST/,
http://www2.ebi.ac.uk/clustalw/.

Table 1. α- Helices of AMA-1 protein.

S.No	Parent/ template	Swiss homology model	Predict protein NPS@GOR4
1	55-60	55-60	55-60
2	64-67	64-67	64-67
3	141-146	141-146	142-147
4	158-167	151-167	151-168
5	271-289	271-289	271-289
6	389-399	389-399	389-399
7	425-429	427-435	425-432

Table 2. β-sheets of AMA-1 protein.

S.NO	Parent/ template	Swiss homology model	Predict protein NPS@GOR4
1	44-48	44-48	43-50
2	78-82	78-82	75-82
3	85-89	85-89	85-90
4	96-98	96-98	94-98
5	100-104	100-104	101-105
6	126-128	126-128	126-130
7	138-140	138-140	135-140
8	182-187	178-183	178-187
9	190-196	186-192	190-194
10	220-224	202-206	205-220
11	231-235	213-217	215-232
12	251-261	233-240	233-245
13	264-266	254-258	254-259
14	272-276	285-291	290-295
15	343-349	294-299	339-349
16	352-357	305-309	351-358
17	363-367	311-317	363-369
18	369-375	323-325	370-375
19	381-383	346-348	379-386
20	414-418	367-369	412-418
21	422-424	376-378	419-424
22	437-439	385-389	437-440
23	446-449	393-397	442-449
24	455-467		452-467

Pair-wise comparison (Bairoch and Rolf, 1997; Bairoch et al., 2004) between all the known amino acid sequences of AMA-1 homologs indicate greater than 50% identity, with 16 cysteine residues conserved in all sequences. All these cysteins are found in the ectodomain of the molecule, which are stabilized by 8 intramolecular disulphide bonds (Figure 2). The ability of this molecule to induce a protective immune response has been shown to be dependent up on a conformation stabilized by disulphide bonds (Hodder et al., 1996). The secondary structure predicted by protein server (Tables 1 and 2) matched almost exactly with the secondary structure of AMA-1 homology model (Figure 3). The over all summary (Guex and Peltsch, 1997) of AMA-1 protein was classified as 24 β-sheets, 7 helices and 27 turns. The results from SWISS MODEL and predict protein almost coincided exactly and also shown a high degree of conservity (Marshall et al., 1989) to the parent /template used.

Energy minimization/computations were done with GROMOS 96 implementation of SWISS-PDB SERVER (Van Gussteren et al., 1996) (Table 3). Further, tertiary structure validation done on the Procheck z-score shown 84.2% resides in the most favored regions, 13.4% in the additional allowed regions, 1.2% in the generously allowed regions and 1.2% in the disallowed regions for

Table 3. Energy computations of AMA-1 protein done with the GROMOS96 implementation of SWISS-PDB viewer.

Parent/template 1w81	Bonds	Angles	Torsion	Improper	Non-Bonded	Electro-static	Constraints	Total
α-Helices kj/mol	1745.248	2390.070	2538.835	411.053	-10144.44	-10643.37	0.0000	-13702.609
β-sheet kj/mol	1064.294	1958.388	2442.315	399.156	-10777.19	-10990.13	0.0000	-15903.167
Coil Kj/mol	275.311	1452.650	2295.883	396.976	-12086.49	-11607.36	0.0000	-19274.029
Generated model of AMA-1								
α-Helices kj/mol	3110.9195	3374.154	1747.296	819.936	-6768.78	-11517.74	0.0000	-14303.335
β-sheet kj/mol	2238.438	3186.691	1832.766	876.545	-1961.06	-11810.04	0.0000	-1715.543
Coil Kj/mol	1252.562	2912.238	1980.121	900.787	-4972.65	-1242.71	0.0000	-10348.65

Computational were done in vacuo with GROMOS96 43B1 parameters set; GROMOS96, W .F.Van Gusteren et al. (1996).

Table 4. Tertiary structure validation of AMA-1 protein.

Checks performed	Z-score of Homology model of AMA-1	Normal Z-score of Parent/Template 1W81
Residue in most favoured regions	84.2%	84.9%
Residue in additional allowed regions	13.4%	13.3%
Residue in generously allowed regions	1.2%	1.2%
Residue in disallowed regions	1.5%	1.2%

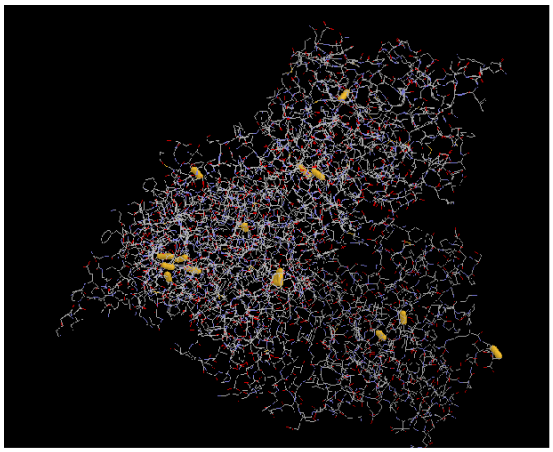

Figure 2. Model showing conservity of 16 cysteine residues of AMA-1 protein.

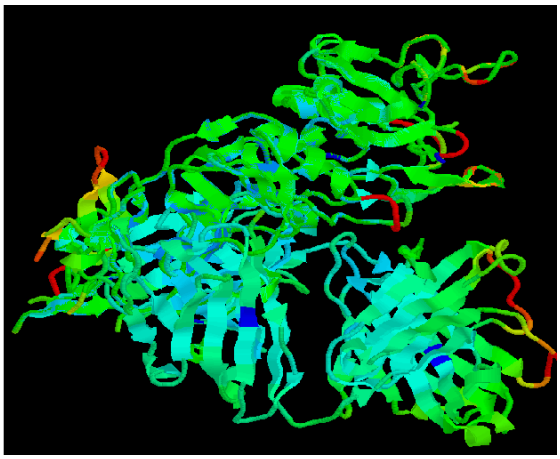

Figure 3. The structure of the protein. Secondary structure validation of AMA-1 protein.

the generated homology model of AMA-1, to the normal Z-score of parent /template shown 83.9% residues in the most favoured regions, 13.3% in the additional allowed regions, 1.2% in the generously allowed regions and1.2% in the disallowed regions (Table 4). The Z-score results of the generated model almost coincides to the Z-score results of the parent/template used, thus proving the model to be almost correct.

Conclusion

Using protein modeling tools, building a homology model for this protein has been accomplished and visualized. In the absence of 3D structure of AMA-1 protein of *P. cynomolgi*, the generated protein model of AMA-1 may be used to understand the antigenic sites and regulatory function by visualizing its domains and structural conformation (Rajesh and Saravanan, 2005; Yogesh et al. 2005). The degree of conservation of AMA-1 sequence and structure implies a conserved function for this molecule across different species of *Plasmodium,* Mary et al., (1996).

Apical membrane Antigen (AMA-1), which is an asexual blood–stage antigen of Plasmodium, is an impor-tant candidate for testing as a component of a malarial vaccine (Crewther et al., 1990; 1996). However, before

using the model for further work, it should be checked for its geometrical, stereo chemical and conformational accuracy before taking up for rational drug design, to avoid later complications.

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