Biochemistry & Physiology: Open Access

Ingale, Biochem Physiol 2014, 3:2
DOI: 10.4172/2168-9652.1000134

Research Article Open Access

Epitopes Identification for Vaccine Design and Structural Aspects of Dengue Virus 3 Envelope Protein

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Rec date: May 19, 2014, **Acc date:** May 28, 2014, **Pub date:** June 04, 2014

Abstract

Dengue is one of the most imperative emerging vector-borne viral diseases. A foremost hitch in designing vaccine for the dengue virus has been the high antigenic variability in the envelope protein of different virus strains. To foster operational vaccines it is essential to target multiple antigenic components of the virus, thus focusing the immune system to protect the host from the virus. Consequently, it is essential to study the structural and functional features of this DENV3 envelope protein in the stoppage of the disease. The purpose of this study was in silico structural characterization of DENV3 envelope protein and to predict their antigenic determinants. This endeavor represents the first structural and epitopes prediction study of DENV3 envelope protein of Indian origin. Computational analyses were performed and a homology model of DENV3 envelope protein was generated. The quality of the model was evaluated by PROCHECK, VERRIFY-3D, PROSA and Errat. Results indicate that 89.88% overall quality of predicted model with a -4.67 Z-score. The model structure was finally submitted in Protein Model Database. The results of MetaPocket server predicted the binding sites, which are good and helpful for docking purpose. The analysis revealed trustworthy conformational B-cell and CTL epitopes that can promote the desired immune response against dengue virus. This information may also help in designing vaccine against dengue in deficiency of experimentally resolved structures.

Keywords: Dengue virus; Vaccine design; Epitopes; Envelope protein; Immunity

Introduction

Dengue virus (DENV) is an arthropod-borne human pathogen that represents a serious public health threat. Dengue virus infection to humans causes a spectrum of illnesses ranging from mild classical dengue fever to severe dengue hemorrhagic fever and dengue shock syndrome. It is one of the most significant universal pathogens and may symbolize a global pandemic [1]. Afterward, malaria DENV is the highest usual mosquito borne pathogen that infects humans. Mosquitoes *Aedes aegypti* transmit DENV to human, well adapted to urban environments. The clinical appearances of dengue virus infection increased from a simple febrile illness to a hemorrhagic fever and a conceivably fatal hemorrhagic shock syndrome [2]. Dengue infection is a main reason of morbidity in tropical and subtropical regions, infects 50 to 100 million people each year, causing almost 40% of the world population at danger and causing more than 20,000 deaths per year, largely in children's [3-5]. This virus is guilty for the premier rate of disease and mortality among members of the Flavivirus genus. The proteins required for the fitness of the virus provide several potential targets against which to develop antiviral drugs. Although several anti-dengue therapies are in clinical studies [6,7] there are currently no approved vaccines to prevent dengue and no antiviral drugs to treat the disease. DENV is a lipid-enveloped positive-strand RNA virus. The RNA genome of DENV is about 10.7 kb and encodes three structural proteins, namely capsid protein, membrane protein, and envelope protein. Besides the structural proteins, there are seven nonstructural proteins (NS), which are associated with viral replication and disease pathogenesis. The coding of the viral proteins is structured in the genome as C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5 [8,9]. The pathogenesis of severe dengue disease remains unclear, but magnitude of DENV replication is believed to be one of the major determining factors. Dengue virus has a relatively simple structure; the envelope protein is the major structural protein exposed on the surface [10]. The structural (envelope E) proteins and non-structural proteins are the dominant sources of cross-reactive CD4+ and CD8+ cytotoxic T- lymphocyte epitopes [11-13]. Some immunization studies show that these proteins are important for inducing protective immunity [14]. The envelope protein can be taken as an ideal target to prevent viral entry or infection of the host cell, or inhibit fusion of the viral envelope with the host vesicles. It can be considered for the development of drugs. The complex web of interactions between the host immune system and the pathogen determines the outcome of any infection. Computational models are proving to be enormously useful in understanding the complex interplay between host and pathogenic factors [15].

Cellular immune responses are recognized to play a role in dengue immunity and the application of T-cell epitopes has been extensively studied as an alternative to vaccines designed for humoral immunity. However, some dengue vaccine formulations were not successful and there remain multiple issues for the development of human T-cell epitope-based vaccines. This study was motivated on the wide-ranging analysis of dengue virus 3 envelope protein sequence data of Indian origin. This model was further analyzed to identify targets for candidate epitope-based T-cell vaccine formulations against all current and possibly future dengue virus infections. The current vaccines are DNA vaccines, peptide vaccines and epitopic vaccines. Epitopes are the small antigenic segments of viral proteins and causes infections in the host. An epitopic vaccine provides more potent and controlled

immune response and eliminates the potential lethal effects of the use of whole viral proteins [16]. Promiscuous epitopes may overcome the population coverage. Secondly, the conserved epitopes reduces antigen escape associated with the viral mutation [17]. So the present study was designed for homology modeling, prediction of CTL epitopes and B cell epitopes and to analyze their antigenic potential.

Material and Methods

Structure prediction and analysis

The primary structure analysis of the DENV3 envelope protein was computed using the Protparam and PEPSTATS online analysis tool [18,19]. Prediction of secondary structure elements was performed using the SOPMA at NPS server and Jpred server [20,21]. It was noticed that the three-dimensional structure of the DENV3 envelope protein of Indian origin was not available in PDB. Therefore, the present study was planned to predict the 3D model and to predict epitopes of DENV3 envelope proteins. Structure template with PDB ID 1UZG having 96.00% identity was selected for the envelope protein. This template was used as a reference to determine the 3D structures of DENV3 envelope protein of Indian origin. Protein structure prediction server MODWEB predicted the homology model based on MODELLER server. The 3D models of DENV3 envelope protein were constructed using the protein structure homology model-building program MODWEB with energy minimization parameters [22]. The modeled tertiary structures were built on the basis of sequence identity with the high-resolution crystal structures of the Dengue virus 3 isolated from Mexico. The CHIMERA viewer [23] was used to visualize and refine the model, and JMOL was used to generate publishable images of the DENV3 envelope protein model. The modeled 3D structures were evaluated and validated with the PROSA; Verify 3D and PROCHECK programs [24].

Prediction of T-cell epitope and B-cell epitope

The computational prediction of T-cell epitope sequences in the conserved regions was accomplished through NetCTL1.2 algorithm (http://www.cbs.dtu.dk/services/NetCTL) and TEPITOPE server. The NetCTL [25] predicts peptides restricted to 12 HLA class I supertypes, combined with predictions of HLA binding, TAP molecules and proteasomal C-terminal cleavage. The parameters used for NetCTL prediction was: 0.15 weights on C terminal cleavage (default), 0.05 weights on TAP transport efficiency (default), and 0.5 thresholds for HLA supertype binding. Moreover, BCPred [26] online server with 75% specificity criteria for epitope prediction was used to predict B-cell epitopes. Transmembrane topology of the envelope protein was checked using TMHMM online tool [27]. The antigenicity of protein was checked using Kolaskar and Tongaonkar method (http://imed.med .ucm.es/Tools/antigenic.pl) and SVMTriP online antigen prediction server [28,29].

Conformational B-Cell epitope prediction

Spatial Epitope Prediction of Protein Antigens (SEPPA) server at the (http://lifecenter.sgst.cn/seppa) was used to predict conformational B-cell epitope. The 3D protein structure predicted by modeler was used as input; each residue in the query protein will be given a score according to its neighborhood residues information. The default values of THRESHOLD were set at 1.80 to help to denote the epitope residues [30].

Ligand binding site prediction

The diversity of algorithms has been developed to identify ligand-binding pockets in protein structures. The accessible methods use geometric criteria, energy functions or a combination of both. Generally most of these methods correctly identify the location of the binding site in 70-90% of the cases if the protein analyzed is in the bound conformation. We practiced MetaPocket 2.0 server (http://metapocket.eml.org) to recognize ligand-binding sites on protein surface. The MetaPocket is a consensus method, in which the predicted binding sites from eight methods: LigsiteCS, PASS11, GHECOM, Q-SiteFinder, POCASA, SURFNET, Fpocket, ConCavity are combined together to improve the prediction success rate.

Results and Discussions

The current study was originated to perform structure based sequence analysis studies on the envelope glycoprotein of Dengue virus 3 isolated from India. The protein sequence was obtained from the NCBI protein database using accession gi|428621155|gb|AFZ40105.1| envelope, partial [Dengue virus 3]. Primary structure analysis revealed that the envelope glycoprotein of Dengue virus 3 (493aa) had a molecular weight of 53665.76 D and theoretical isoelectric point (pI) of 6.83. An isoelectric point below 7 indicates a negatively charged protein. The instability index (II) is computed to be 29.81. This categorizes the protein as stable. The aliphatic index showed to be 86.21. The N-terminus of the sequence is reflected to be M (Met). The negative Grand average of hydropathicity (GRAVY) of -0.094denoted that the protein was hydrophilic. The amino acid Gly (G), Val (V), Thr (T), and Leu (L), were found in rich amounts in the protein. Secondary structure revealed that it had 21.10% alpha helices, 7.71%, beta turns, 33.06% extended strand and 38.13% coils (Figure 1). Transmembrane topology of the DENV3 Protein was checked using TMHMM online tool reveals that two transmembrane helices are present in protein (Figure 2). The TMHMM online server showed that residues 1–442 presented outside region, residues 466–471 were within the transmembrane and residues 443–491 were inside the region of the protein. Hydropathy analysis of DENV3 envelope proteins by the TOPCONS single [31] Signal P-4.0 [32], and TMHMM programs also suggested the presence of two TM helices, depending on the program used and the species considered.

Model function and validation

To determine the possible function of DENV3 Protein, the sequence was subjected to comparative protein structure modeling using the target protein sequence as query for MODELLER server described in materials and methods. Significant hits were obtained for the MODWEB server, which retrieved the crystal structure of the DENV3 envelope protein from Dengue virus of Mexican origin (PDB ID: 1UZG). The alignment coverage region for target residue (1-392) showed the 96 % sequence identity with template 1UZG residue 1–392. The 3-D structure of an envelope protein was developed through homology modeling by using from the crystal structure of Dengue virus 3 isolated from Mexico PDB ID: 1UZG Chain A, B, at 3.5 angstrom resolution [33] as template. The Comparative modeling of DENV3 protein was accomplished using a restrained-based approach implemented in MODELLER9v6 [34].

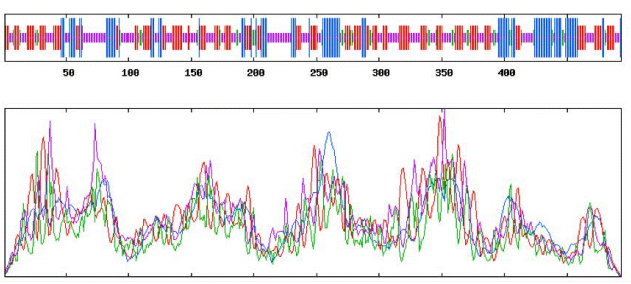

Figure 1: Secondary structure prediction of DENV3 envelope protein

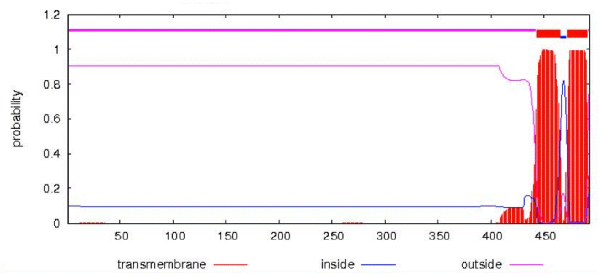

Figure 2: Transmembrane topology of DENV3 envelope protein: red line indicates a transmembrane helix in protein sequence, blue line inside show amino acid residue and pink line shows outside AA residue.

A bunch of three models for target protein was constructed. The resulting three-dimensional models of DENV3 Protein were sorted according to score calculated from discrete optimized protein energy (DOPE) scoring function. The final model that shared the lowest Root Mean Square Deviation (RMSD), relative to the trace (Ca atoms) of the crystal structure was selected for further studies. The validation of model was performed by accessing the quality of backbone conformation of model by PROCHECK for reliability. The perceived Ramchandran plot (Psi-Phi) pairs had, 89.9% of residues in (A, B, L) most favored regions, 10.5% core residues in (a, b, l, p) additional allowed regions, no residues in generously allowed regions and no residues in disallowed regions as shown in Figure 3 and above values indicated a good quality model. Whereas the crystal structure of dengue virus from Mexico PDB ID 1UZG shows the 89% residue in most favour region. In order to characterize the model, structural motif and mechanistically important loops were assigned to build the final 3D model of DENV3 protein. The 3D model of this protein using the template 1UZG consisted of two domains and encompassed 6 beta-sheets and coils (Figure 4).

Verify3D and ERRAT were also used to further assess the quality of the DENV3 envelope protein model. Verify3D analyzes the compatibility of the model against its own amino acid sequence and results revels that 92.38% of the residue had an average 3d-1D score 0.64 (Figure 5). However, the overall quality score is evaluated by ERRAT and PROSA was found to be 89.88% and -4.67 respectively (Figures 6 and 7). It indicates that the predicted model is of good quality. The modeled protein was submitted to protein model database and can be downloaded with the ID PM0079147.

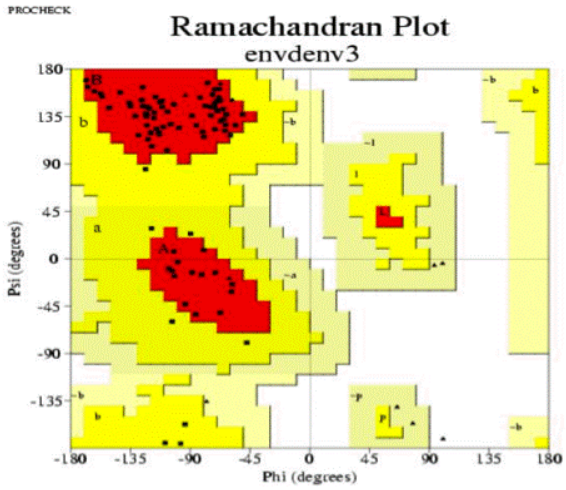

Figure 3: The Ramchandran plot for the modeled DENV3 envelope protein. 89.9% of residues are found in the most favored regions, 10.5% of residues are in the most allowed regions and no residue are found in the outlier regions. The plot was created with PROCHECK program.

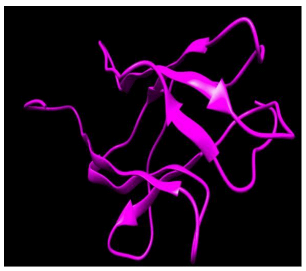

Figure 4: Predicted 3D structure of the DENV3 envelope. The model was generated with MODWEB using PDB template 1UZGA. CHIMERA was used to visualize the model.

Epitope prediction of protein antigens

Using SEPPA server we predicted potentially immunogenic regions of DENV3 envelope protein. On the basis of different propensity scores and solvent accessibility, this server analyses 3D structures and aim at the division of antigens surface in epitopic and nonepitopic patches; they all rely on training datasets comprising resolved antibody and antigen complexes [35]. There are fifty-two numbers of epitopes were predicted from (Chain B) of 104 aa using default threshold value 1.80. The predicted epitope are be visualized with JMOL in different renderings in Figure 8. In this structure, tints from blue to red represent a rising antigenicity. Highlighted epitope residues predicted are shown in red solid spheres. The prediction results are also displayed in a table, residues are listed sequentially. The predicted

epitope residues are highlighted in yellow. The core residues are shown in lowercase.

Cytotoxic T- Lymphocyte epitopes

Epitope predictors are consistently experienced on large sets of epitopes resulting from various pathogens. We examine all promising peptide fragments of 9 amino acids within a specific protein, and exclude those fragments that cannot be appropriately processed by either the proteasome, TAP or the MHC class I molecules.

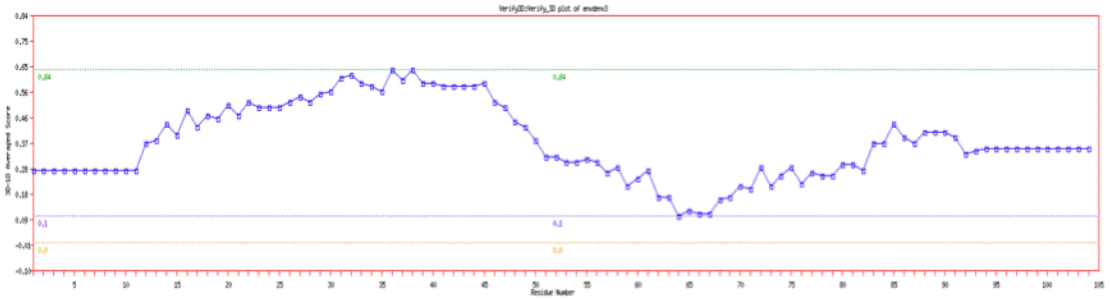

Figure 5: Verify 3-D graph of model structure of DENV3 envelope protein

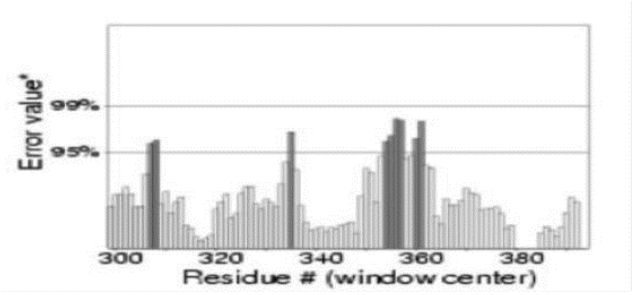

Figure 6: Errat value of DENV3 envelope protein

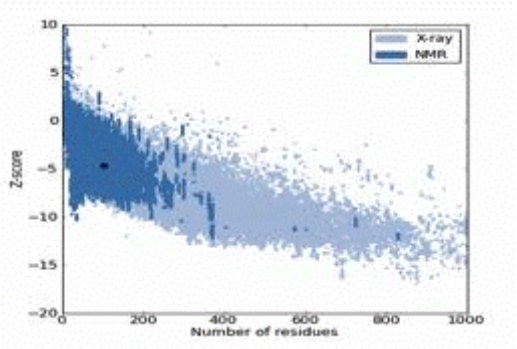

Figure 7: Shows the PROSA Z-score to validate quality of model

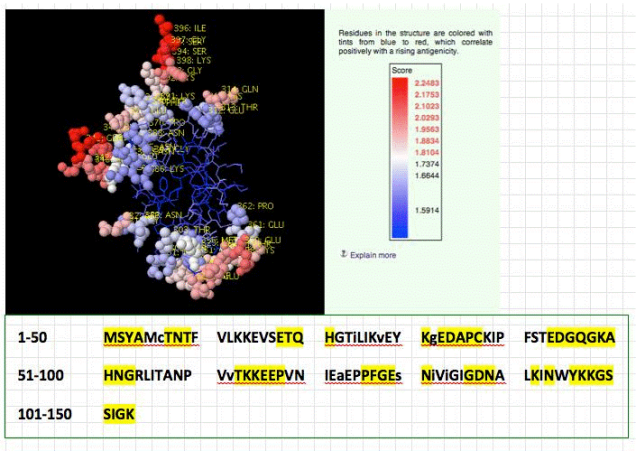

Figure 8: Antigenic epitope sites predicted by using SEPPA server. The red sphere shows highest antigenicity residue and blue are low antigenic. The yellow residues are predicted epitope.

CTL epitopes prediction results of DENV3 envelope protein reveals that 10 MHC ligand are found in sequence having high score e-values are position at 124 PIEGKVVQY132, 129 KLELKGMSY137, 168ITEAILPEY176, 204WMVHRQWFF212, 273GTSIFAGHL281,289 KLELKGMSY297, 300CTNTFVLKK 308, 310VSETQHGTI318,316GTILIKVEY 324 and 478 IAIGIITLY 486. These are the immunodominant epitopes restricted by MHC class I located indiscriminately in protein sequence. This data indicate CTL epitopes in DENV3 envelope protein are randomly distributed, and that this distribution is similar to the distribution of CTL epitopes in proteins from other proteomes.

Antigenicity prediction

We applied Kolaskar and Tongaonkar antigenicity method, to predict antigenic peptides that bind to the MHC molecule are the first bottleneck in vaccine design. Figure 9 shows the antigenic determinant plot indicating sequence number on x-axis and average antigenic propensity on y-axis. Average antigenic propensity for DENV3 envelope protein is 1.0247. There are 20 antigenic epitopes in overall sequence (Table 1). The highest peak at start position 244KKQEVVVLG52 amino acid residues, 87DQNYVCKHTY96 amino acid residues and 17GATWVDVVLEHGGCVT32 amino acid residues.

The average for the whole protein is above 1.0; all amino acid residues above 1.0 are potentially antigenic. The highest peak sequence of antigenic determinant site is used for insertion. Highest peak in antigenic determinants plot indicate antigenic site for attachments at position 244-252 aa residues. Predictions are founded on a table that imitates the existence of amino acid residues in experimentally known segmental epitopes. The capacity of an individual antibody-combining site to react with only one antigenic determinant and the aptitude of a population of antibody molecules to react with only one antigen [36].

B-cell epitope prediction

The prediction of B-cell epitopes in an antigen sequence is chief and intricate problem. Although most antigenic determinants of proteins are discontinuous, it is possible to mimic epitopes by synthetic peptides [37]. B-cell epitopes are substantial for protection against virus infection. It plays a dynamic role in the development of peptide vaccines, in diagnosis of diseases, and also for allergy research [38]. B-cell epitopes were predicted using BCPred having the conditions of length 20 and 75% specificity using BCPred algorithm. The B-cell epitopes were mapped on the protein along its amino acid sequence. The B-cell epitopes predicted by BCPred server in dengue virus 3 envelope proteins are shown in Table 2. The predicted peptides are displayed rank- wise based on scores obtained by the trained recurrent neural network. Ten epitopes were predicted and all of them were exposed outside the membrane. The highest score is at position 351-371 is highly immunogenic. Epitopes with antigenic properties can be important in raising the desired immune responses.

Ligand binding sites prediction

We used MetaPocket 2.0 for ligand binding site prediction by pocket detection. MetaPocket 2.0 is provided with the target protein's crystal structure, the top-ranked MODWEB templates and theoretical protein models generated by MODELLER. In this situation, we consider the top four identified binding pockets for further analysis (Figure 10). The first MetaPocket site (MPK 1) consists of six packet sites, the first pocket from (GHE-1), the first pocket from (LCS-1) and PCS1, the second pocket from (FPK-2), the first pocket from PASS (PAS-1) and the first pocket from Concavity (CON-1) with total z-score 11.06 and size of 7. The second MetaPocket site (MPK 2) consists of 3 pockets; from SNF-1, LCS-2, GHE-2 and the total z-score is 4.04 size 3. The third MetaPocket site (MPK 3) consists of 6 pocket, [LCS-3, GHE-3, FPK-1, QSF-3, PCS-2, SFN-3] with total z-score 1.55 and size of 6.

The fourth MetaPocket site (MPK 4) consists of 2 pocket, PCS-3, SFN-2with total z-score 1.55 and size of 2. The Figure 11 shows exact residue location of the potential binding sites from a prediction DENV3 envelope protein. The header binding sites 1, 2, 3 and 4 are designated for MetaPockets 1, 2, 3 and 4 respectively. In the case above, potential binding sites of four MetaPockets are given; they are shown in residue format with each line starting with 'RESI'. Shown residue below is constructed in three parts: residue name, chain indicator and residue sequence number.

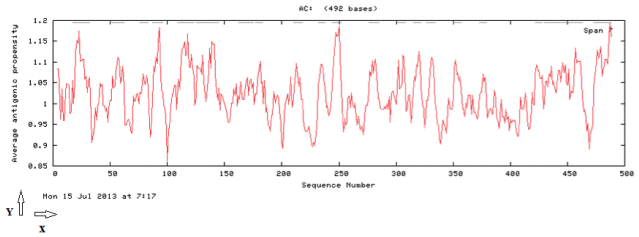

Figure 9: Antigenic determinant plot of DENV3 envelope protein

Sr. No.	Start Position	Sequence	End Position
1	17	GATWVDVVLEHGGCT	32
2	50	ATQLATLRKLCIE	62
3	76	TQGEA VLP	83
4	87	DQNYVCKHY	96
5	109	GKGSLVTCAKFQCLE	123
6	125	IEGKVVQYENLKYTVIITVHT	145
7	162	ITPQASITEA ILPE	175
8	177	GTLGLEC	183
9	210	WFFDLPLPW	218
10	232	KELLVTF	238
11	244	KKQEVVVG	252
12	276	IFA GHLKCRL	285
13	301	TNTFVLKK	308
14	315	HGTILIKV	322
15	327	EDAPCKIP	334
16	350	ITANPVVT	357
17	373	ESNIVIG	379
18	422	SVGGVLN	428
19	430	LGKM VHQIFGSAYTALFSGVSWVMKIGIGVLLT W	463
20	473	MSFSCIAIGIITLYLG	488

Table 1: Antigenic sites of DEN V3 envelope protein predicted by Kolaskar and Tongaonkar method (1990)

Conclusions

In summary, we have provided the first 3 D structure for DENV3 envelope protein of Indian origin. For designing effective inhibitors against envelope proteins, it is important to have knowledge of sequence and structure of protein.

Position	Epitope	Score
351	TANPVVTKKEEPVNIEAEPP	1
379	GIGDNALKINWYKKGSSIGK	0.996
240	NAHAKKQEVVVLGSQEGAMH	0.993
62	EGKITNITTDSRCPTQGEAV	0.963
93	KHTYVDRGWGNGCGLFGKGS	0.953
459	VLLTWIGLNSKNTSMSFSCI	0.952
311	SETQHGTILIKVEYKGEDAP	0.948
25	LEHGGCVTTMAKNKPTLDIE	0.863
123	EPIEGKVVQYENLKYTVIIT	0.824
174	PEYGTLGLECSPRTGLDFNE	0.739

Table 2: Predicted B cell epitopes with residue position and their scores.

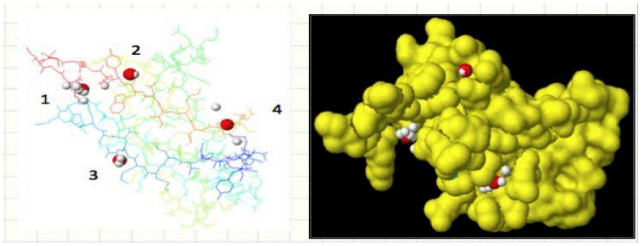

Figure 10: Show the predicted potential binding sites in DENV3 envelop protein

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------------------------------------------------------------------------------
HEADER binding site ID: 1
RESI      LYS_B^392^    GLY_B^393^    SER_B^394^    GLN_B^314^    PHE_B^371^
RESI      HIS_B^315^    LYS_B^391^    GLY_B^316^    SER_B^395^    LYS_B^398^
RESI      GLY_B^397^    GLU_B^312^    THR_B^313^    TYR_B^390^    TRP_B^389^
RESI      ASN_B^388^    ASN_B^346^    ARG_B^348^    GLY_B^347^    GLU_B^368^
RESI      PRO_B^370^    PRO_B^369^    ILE_B^396^    VAL_B^310^
HEADER binding site ID: 2
RESI      ASN_B^346^    GLY_B^347^    ARG_B^348^    GLU_B^368^    PRO_B^369^
RESI      PRO_B^370^    LYS_B^391^    GLU_B^312^    THR_B^313^    GLN_B^314^
RESI      HIS_B^315^    GLY_B^316^    LYS_B^392^    GLY_B^393^    SER_B^395^
RESI      LYS_B^398^    TYR_B^390^    PHE_B^371^
HEADER binding site ID: 3
RESI      MET_B^299^    LYS_B^332^    ILE_B^333^    ASN_B^353^    PRO_B^354^
RESI      PRO_B^334^    GLU_B^309^    SER_B^311^    LEU_B^319^    ILE_B^320^
RESI      LYS_B^308^    VAL_B^310^    LYS_B^321^    ASN_B^364^    ILE_B^365^
RESI      GLU_B^366^    THR_B^351^
HEADER binding site ID: 4
RESI      SER_B^336^    GLU_B^338^    VAL_B^377^    GLY_B^379^    ILE_B^380^
RESI      GLY_B^381^    ALA_B^384^    CYS_B^300^    THR_B^301^    ASN_B^302^
RESI      PRO_B^334^    PHE_B^304^    THR_B^303^    ILE_B^378^    ASN_B^383^
RESI      ASP_B^382^
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Figure 11: Shows the predicted binding sites residues location.

Bioinformatics analysis has open new vistas to provide more insights into protein sequence and structural features. In silico analysis of dengue virus pathogen structure and epitopes also allows us to expose novel interactions of relevance for understanding pathogenesis, host response, all of which can be applied the development of novel vaccines and immune therapeutics. Computational tools enable researchers to move rapidly from genomic sequence to vaccine design. Accordingly, discovering novel putative targets through the comprehensive epitope information may provide valuable novel insights for developing novel drugs and vaccines against dengue virus. B-cell and CTL epitopes are important in raising the desired immune responses. As knowledge of epitopic regions on protein is important in designing effective inhibitors, therefore, both B-cell and T-cell epitopes were predicted that were well conserved in the DENV3 Envelope protein. These predicted epitope sequences are potential vaccine candidates, but functional as well as biological assays should be performed to verify whether they are indeed appropriate to be included in a vaccine formulation. Vaccine design can be expedited via the application of in silico techniques combined with immunological methods.

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