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Research Article

DEVELOPMENT OF DENGUE DATABASE BY USING BIOINFORMATICS TOOLS AND TECHNIQUES

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ABSTRACT

Dengue database is an effort to collect information about the dengue virus, dengue virus genes, dengue virus drugs targets and drugs, disease into a central portal to facilitate dengue researchers from several disciplines such as drug discovery, vaccine development, epidemiology and comparative genomics. The database also serves as link to Global Sequence Information eg. NCBI, DDBJ, EMBL, PDB, Expasy, Medical Literature like PubMed etc. The main aim of this project is to create a web enabled database for the Dengue Disease drugs targets and provide most useful information Like Genomic study, gene Information, Drug target information for the researcher. The Dengue genome encodes a single large open reading frame that is translated to form a viral polyprotein. The dengue polyprotein is cleaved by viral and host proteases to produce 3 structural proteins and 7 non-structural (NS) proteins. We have modeled all structural and nonstructural proteins by using bioinformatics tools and techniques and presented in this paper.

INTRODUCTION

Bioinformatics

Bioinformatics is a newly developed interdisciplinary research area lying at the interface of chemical, biological and computational sciences. It can be used to solve biological problems and for gaining an understanding of the molecular basis of biological phenomena. The information so obtained is used in analyzing protein behaviors, designing experiments to know the relationship between structure and function, obtaining detailed understanding of molecular processes and developing drugs based on chemical similarity of known drugs. Certain processes in drug development such as Lead identification, Quantitative Structure Activity Relationship (QSAR), etc. for the development of new potent drugs can be done easily via the available bioinformatics tools which is highly cost-effective and eco-friendly.

There is a huge library of data collected from biological sources. Managing these fast-growing data is very difficult and hence nowadays they are being stored and exchanged in digital form as files or databases. Some primary nucleotide sequence databases are the DDBJ, EMBL nucleotide database and the GenBank. Ensembl is a well known genome database. Renowned protein sequence databases are the Uniprot, Swiss-prot and the PIR. Protein structure databases include the PDB, CATH, SCOP, Swiss-Model and the ModBase.

Structure Based Drug Design and Prediction of Protein Structure

The Swissprot database consists of about 356194 protein sequences. In contrast, the RCSB PDB has only 49295 protein structures deposited for the same date. This is due to the difficulties faced in the experimental methods such as the crystallization and phase problems in XRD and resolution problems in NMR. Structure based drug design requires considerable knowledge of the three-dimensional structure of the receptor protein.

Homology Modeling In Bioinformatics

In homology modeling, the computer is used as an experimental tool to predict the 3D structure of an unknown protein from its aminoacid sequence. The basis of this is to build suitable models that will presumably closely resemble the unknown protein and to evaluate the quality of the models and choose the best one amongst the built models. Homology means ancestral relationships, and assumes that proteins from the same families share folding motifs even if they don't share the same sequences (Bergeron B, 2002). The models are based on known protein structures (templates) which resemble the

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unknown amino acid sequence. Protein structures are more conserved than protein sequences and hence noticeable levels of sequence similarity usually implies significant structural similarity (Marti-Renom *et al*., 2000). The sequence alignment and template structures determine the quality of the model thusproduced. Complications during model building are usually caused by the structure gaps (missing aminoacid residues) present in the template which arise from poor resolution in NMR and XRD experiments. A decrease in sequence identity lowers the quality of the model. There is a twilight zone limit of sequence identity between the template and target protein at which point the homologous template backbone is no longer sufficient to constrain the correct packing of the buried side chains (Chung *et al*,.1996).

Homology models can be used to formulate hypotheses about the structure biochemistry of the query sequence but they have to be accompanied with experimental results in order to prove such hypotheses. Critical Assessment of Techniques for Protein Structure Prediction (CASP) is a biannual large-scale experiment which is used to assess the accuracy of models built by this technique. The basis for homology modeling is the Protein Data Bank (PDB) that contains 3D structures of proteins determined by experimental methods such as XRD and NMR.

The general procedure for homology modeling is:

1. Search for crystal templates of high similarity and high evolutionary relationship with regard to secondary structure and three dimensional correspondence.
2. Align the sequence of the desired protein to the crystal structure sequence
3. Build homology models using a comparative modeling software
4. Evaluate the models of the basis of stereochemistry parameters.

In general, the models should be based on templates with as high sequence similarity and conserved secondary structure as possible. Templates with 30% sequence similarly have been named as a lower limit for reasonable accuracy in the final model. It is generally accepted that the higher the sequence identity between the template and the target, the better will the model turn out.

Architecture of Dengue Virus

Flaviviridae are a family of around 66 viruses, of which most of them have been associated with human disease,The most important mosquito-borne pathogen amongst humans is the Dengue virus which is one of the most widespreading diseases in the world.There are four main serotypes of the dengue virus namely DEN1, DEN2, DEN3 and DEN4. The immune system is unable to respond to the stronger infection by the second strain as a result of which the secondary infection becomes far more serious. Hence, it is very difficult to find a vaccine that would protect effectively against all four dengue serotypes. Flaviviruses consist of a single stranded positive sense RNA genome that is approximately 11 kb in size. The viral genome is translated as a polyprotein in the cytoplasm. There are signal and stop-transfer sequences that direct the translocation of the polyprotein back and forth across the endoplasmic reticulum (ER) membrane. The polyprotein is subsequently co- and post-translationally modified by viral and host-encoded proteases to produce three structural and seven nonstructural proteins. The mature virion contains three structural proteins: the capsid, C; a membrane associated protein (which is produced from the precursor prM), M; and the envelope protein, E. The nonstructural (NS) proteins include large, highly conserved proteins NS1, NS3, and NS5 and four small hydrophobic proteins NS2A, NS2B, NS4A, and NS4B(Chambers, T. J., *et aI*, 1990, Henchal *et al*.,1990 and Zhang *et al*.,2003).The following diagram depicts the Dengue genome organization (Fig 1.)

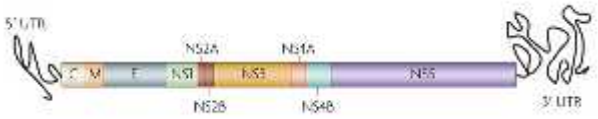

Fig-1 Dengue virus genome

The dengue virus genome encodes three structural (capsid [C], membrane [M], and envelope [E]) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins.

The core of the virus is the nucleocapsid, a structure that is made of the viral genome along with C proteins. The nucleocapsid is surrounded by a membrane called the viral envelope, a lipid bilayer that is taken from the host. Embedded in the viral envelope are 180 copies of the E and M proteins that span through the lipid bilayer. These proteins form a protective outer layer that controls the entry of the virus into human cells.

The 495-amino-acid (aa) envelop (E) glycoprotein, one of the three structural proteins, is the principal component of the external surface of the virion (Chang G J,1997), and it is responsible for a wide range of biological activities, including binding to host cell receptors, fusion to and entry into host cells, therefore, this protein directly affects host range, cellular tropism, and, in part, the virulence of the virus (Kuhn *et al*, 2002; Roehrig *et al*,1997). Furthermore, the E protein also stimulates host immunity by inducing protective and neutralizing antibodies (Chang G J, 1997).

Capsid protein

The Dengue capsid protein has a molecular weight of about 13.5 kDa. It is highly basic, being rich in lysine and arginine residues and forms a structural component of the nucleocapsid34. The terminal hydrophobic signal sequence is cleaved by the viral NS2B-NS3 protease before virion assembly resulting in the mature form of DENV C protein (Yamshchikov, *et al*., 1994; Amberg, S., *et aI*,1994). The DENV C protein is necessary for encapsulation of the viral genome. Infected cells release subviral particles that do not contain either C or the viral genomic RNA, suggesting an important role for C in proper packaging of the infectious virion (Ferlenghi, *et al*, 2001). Recently, the structure of the DEN2V C dimer was elucidated by nuclear magnetic resonation (NMR) techniques (Jones, C. T., *et al*, 2003; Ma, L., *et aI*, 2004)). Dengue capsid protein contains four alpha helices and forms a dimer in solution.

Premembrane and Membrane protein

The prM protein is a glycoprotein that forms heterodimers with E protein on the intracellular immature virion surface. The dimerization of prM and E is assumed to prevent exposure of the fusion peptide within the cell. The prM protein contains a stop transfer sequence and a signal sequence in two transmembrane helices.Upon maturation and release of the virion from the cell, the host enzyme, furin, mediates the cleavage of prM to produce M. This process is essential for virus morphology, viral release, and viral infectivity as it releases E from prM. The E proteins then reorganize into homodimer. The mature virion has a smooth appearance compared to the immature virion which contains spikes on the surface (Zhang, W *et al*., 2003).

Envelope protein

Dengue envelope protein with a molecular weight of 54.5 kDa is folded largely into beta-sheets and contain three distinctive domains: the N-terminus central domain (domain I); the fusion (or dimerization) domain that contains the fusion peptide (domain II); and the immunoglobulin (IgG) like domain (domain III) (Crill *et al*., 2001). The domain III is thought to bind to the cellular receptor (Mukhopadhyay, S, *et al*., 2005). Envelope protein appears to be a homodimer in solution and is located on the surface of the viral particle. Exposure of envelope protein dimers to low pH in early endosomes after viral uptake results in irreversible conformational change of envelope protein to a trimer conformation (Heinz, F. X., *et al*., 2004; Kimura, T, *et al*.,1998; Modis, Y *et al*.,2003).

Nonstructural Proteins

The seven nonstructural proteins, NSl, NS2A, NS2B, NS3, NS4A, NS4B, and NS5, are encoded in the 3' region of the viral genome (Fig 1).

NS1 protein

The NS1 protein with molecular weight 38.7 kDa contains a signal sequence that is located in the carboxy terminus of the E protein and is inserted into the ER50.NS1 is a glycoprotein that forms homodimers and interacts with membranes (Smith*et al*., 1985; WinkleretaI., 1989). Glycosylation of NS1 protein isimportant for its dimerization (Pryor, M. J *et al*.,. 1994).

NS2A and NS2B proteins

NS2A protein with a molecular weight of 20 kDa has been identified as a hydrophobic protein with several transmembrane domains and was found to be involved in proteolytic cleavage of NS1 at its carboxy terminus (Falgout *et al*., 1989).The NS2B protein associates with NS3 to form the viral protease.

NS3 protein

NS3 is a nonstructural protein with a molecular weight of 70 kDa. It is hydrophobic and performs many functions. It functions as a serine protease (Biedrzycka, *et al*.,1987; Falgout, B., *et aI* 1991; Li *et al*.,1999), RNA helicase (Wu *et al*.,2005), nucleotide triphosphatase and RNA 5´- triphosphatase (Bartelma, *et al*., 2002; Bartholomeusz, *et al*.,1993; Cui, *et al*.,1998). The N-terminal sequence of NS3 shares homology with other trypsin-like serine proteases

NS4A and NS4B proteins

The small proteins NS4A and NS4B have not been studied extensively. NS4a and NS4b are both hydrophobic proteins whose functions are still not clear. It is suggested that NS4a and NS4b may be involved in viral replication (Salonen, *et al*., 2005).

NS5 protein

The NS5 protein whose molecular weight is around 150 kDa is the largest of the dengue proteins and is highly conserved among the flaviviruses (Mandl, C., *et aI*., 1989). NS5 has a RNA-dependent RNA polymerase activity containing a Gly-Asp-Asp (GDD) domain.

Development of Dengue database

Here comes the bioinformatics come to solve the management problem of database and easy retrieval of data. The main purpose of this project is to provide an online database for researchers and for those who wish to work on Dengue Disease. All the data are linked with GenBank (NCBI) files or other Sequence Servers for the reference. Dengue database is an effort to collect information about the dengue virus, dengue virus genes, dengue virus drugs targets and drugs, disease into a central portal to facilitate dengue researchers from several disciplines such as drug discovery, vaccine development, epidemiology and comparative genomics. The database also serves as link to Global Sequence Information eg. NCBI, DDBJ, EMBL, PDB, Expasy, Medical Litrature like PubMed, etc

Knowledge of the structural information of the Dengue envelope proteins is required in order to gain an understanding of the entry of this virus into host cells and for the design of suitable inhibitors of this viral protein. In addition, the modeled structures would highlight possible regions of the E protein that can be targeted to block viral entry. The design of small molecules capable of inhibiting the viral replication is mandatory and they should be designed such that they display increased bioavailability. The structure of Envelope proteins of Dengue virus has been studied extensively and was found tohave three antigenicdomains I, II and III. Potential target sites were identified in the non structural proteins as well. The unknown structures we remodeled by homology modeling using the Modeller software and the target sites were identified.

The main aim of this project is to create a web enabled database for the Dengue Disease drugs targets and provide most useful information Like Genomic study, gene Information, Drug target information for the researcher,

MATERIALS AND METHODS

The Dengue virus proteins were identified from Expasy server (http://ca.expasy.org/sprot/). The amino acid sequences of the target dengue proteins were obtained from Expasy server. Multiple sequence alignment was done using ClustalX software. In homology modeling, the models were built using the program Modeller. Models were built for each of the proteins and the best model was chosen based on the molpdf and DOPE scores in corporated in the Modeller program. The model with the lowest DOPE and molpdf scores were chosen in each instance.

The next step was the optimization of bond geometry and the removal of unfavorable non-bonded contacts. This process is termed as energy minimization and the number of minimization cycles should be kept at a minimum just sufficient to improve the stereochemistry of the model. This is because, excessive energy minimization will cause the model to deviate markedly from the original model, which is not suitable and should be avoided. The models were evaluated in terms of stereo chemical qualities with the help of Ramachandran plots drawn using the PROCHECK software. This minimized structure, whose quality was evaluated with PROCHECK, was taken as the final model.

As can be seen from the Ramachandran plots (Appendix), most of the residues in all the seven models were in the favorably allowed regions and there were only about 0.6% residues in the disfavored region. This implies that there is a very high probability of these homology models being correct. The correctness of the models was further verified by the G-factor and Verify3D program.

RESULT AND DISCUSSION

The database was designed and implemented. It will be hosted on the internet. Anybody can access this website free of cost. In the HOME page (Figure-2) there is a brief discussion about Dengue virus and its genome structure.

Fig-2 Dengue database homepage

There will be a menu at the top which contains, Home, About us, Databases, Contact and More. There are links provided for Dengue virus genome data in this place as DENV1, DENV2, DENV3 and DENV4 (Figure-3).

Fig-3 Hyperlinks in Dengue database

Through the option Contact, the visitor can get in touch with the team and they can interact with us. More option contains a list services such as, publications, resources, people and research. The option people contain the list and details of the people who have worked for the development of Dengue database.

A database consists of an organized collection of data for one or more multiple uses. As of 2010 the relational model occurs most commonly. Other models such as the hierarchical model and the network model use a more explicit representation of relationships.

In homology modeling, the models were built using the program Modeller. Models were built for each of the Dengue proteins and the best model was chosen based on the molpdf and DOPE scores incorporated in the Modeller program. The models were evaluated in terms of stereo chemical qualities with the help of Ramachandran plots drawn using the PROCHECK software. The correctness of the models was further verified by the G-factor and Verify3D program.All dengue protein models were arranged with sequence and structure in different columns (figure-4 and 5)

Fig-4 Modeled Structures of proteins of dengue virus DENV1

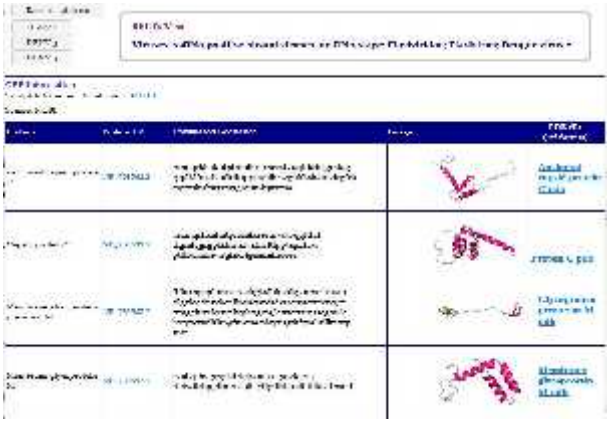

Fig-5 Modeled Structures of proteins of dengue virus DENV2

CONCLUSION

Dengue fever is a disease caused by the Single-stranded RNA flavivirus. It is spread by Aedes Mosquitoes and is endemic to the tropical regions. The virus circulates as four immunologically distinct serotypes. Despite this diversity dengue fever is normally characterized by similar symptoms: fever, joint pain and vascular leakage. It is currently classified as an emerging or re-emerging infectious disease by the WHO. Dengue fever (DF) and dengue haemorrhagic fever (DHF)/dengue shock syndrome (DSS) occur in over 100 countries, with more than 2.5 billion people at risk and an estimated 50 million infection per year. The virus was able to make this genetic transformation due to three mutations that have been discovered in the tail of the dengue viral genome. Through these mutations the viral strain was able to suppress the human antiviral response. This allowed the newly emerging strain to spread, infect more mosquitoes and thus infect more people.

The study is important because it provides a new understanding as to how the virus mutates and this insight might offer ways to

combat the virus. Studying the virus at the genetic level also provides information about which stains of the virus are most likely to trigger epidemics. This information is useful in dengue surveillance. Bioinformatics is the application of computer technology to the management of biological information. Computers are used to gather, store, analyze and integrate biological and genetic information Recent years have seen an explosive growth in biological data. It should be managed and stored for various purposes. The bioinformatics come to solve the management problem of database and easy retrieval of data. The dengue database is an online database for researchers and for those who wish to work on Dengue Disease. All the data are linked with GenBank (NCBI) files or other Sequence Servers for the reference.

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