Journal of Computer Science & Systems Biology - Open Access

JCSB/Vol.3 Issue 1

Research Article | OPEN ACCESS Freely available online | doi:10.4172/jcsb.1000047

Comparative Modeling Study of the 3-D Structure of Small Delta Antigen Protein of Hepatitis Delta Virus

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Abstract

Delta hepatitis is pandemic worldwide, which is caused by Hepatitis delta Virus (HDV) an RNA virus. HDV causes either co-infection or super infection with Hepatitis B virus. Small delta antigen protein of HDV is obligatory for replication of virus. Since it plays a crucial role in virus life cycle, it may be a suitable target for drug development. Three dimensional structure of protein is of great significance for the rational design of many different types of biological experiments. In current study 3-D modeling of small delta antigen protein was performed by using GenThreader followed by modeller9v7. The validation of predicted 3-D structure was done using PROCHECK, Anolea, Gromos96 and Swisspdb-viewer tools. CASTp was used further to study surface features and functional binding pockets in protein. The resulting 3-D model can be used to develop novel inhibitor against small delta antigen protein to cure the disease.

Keywords: Delta hepatitis; 3-D structure; Modeling; Energy minimization; Small delta antigen; Drug development

Introduction

Delta Hepatitis currently infects about twenty million people worldwide (Taylor, 2006) which is most common among populations using injectable drugs particularly in countries bordering the Mediterranean Sea while least common in Eastern Asia, but is also present in Taiwan, China and India. Most children with Delta Hepatitis have been identified in Italy and Greece, with a few in northern Africa. The disease is caused by HDV. The virus was discovered in 1977 by Rizzetto and colleagues while they were studying liver biopsies of patients with hepatitis B surface antigen (HBsAg)-positive chronic liver disease (Rizzetto et al., 1977).

HDV is a subviral satellite of hepatitis B virus, on which it is dependent for its envelope proteins (Lai, 1995). The genome of HDV, the smallest among animal pathogen, is a single-stranded negative sense circular RNA of about 1,700 nucleotides in length that forms a highly base paired rodlike structure (Taylor, 1992). Genome has single open reading frame that is encoded within a single protein the delta antigen protein (dAg). There are two forms of the delta antigen. The small form (195 amino acid long) is essential for HDV replication, and the large form, with a 19-amino-acid extension at the carboxyl end, (214 amino acid long) is crucial for the packing virions.

Assembly of hepatitis delta virus (HDV) in infected human hepatocytes involves association of the single-stranded genomic RNA with multiple copies of both small and large forms of the delta protein (delta Ag) to form a ribonucleoprotein particle which in turn interacts with envelope proteins of the natural helper virus, hepatitis B virus subsequently, for initiation of a new round of replication (Gudima et al., 2002).

During HDV replication, three HDV RNA species are produced: the 1.7-kb antigenome, the 1.7-kb genome, and the 0.8-kb antigenomic-sense RNA. The former two RNA species form circular RNA and represent the replication products of the HDV RNA genome. The 0.8-kb RNA, however, is polyadenylated and thus resembles cellular pol II transcripts. This RNA is the mRNA for translation of HDAg (Lo et al., 1998). In the HDV-infected cells, small (S-HDAg) and large (L-HDAg) both forms of HDAg are found (Bergmann and Gerin, 1986; Bonino et al., 1986; Pohl et al., 1987; Roggendorf et al., 1987). Both forms are translated from the same open reading frame present on the 0.8-kb mRNA; the large form results from an RNA editing event (Casey and Gerin, 1995; Polson et al., 1996; Polson et al., 1998) extending the S-HDAg open reading frame by 19 amino acids to encode the L-HDAg. The S-HDAg is required for HDV RNA replication in vivo (Kuo et al., 1989). In contrast, the L-HDAg inhibits HDV RNA replication (Chao et al., 1990; Glenn and White, 1991).

Bioinformatics approaches are successfully being applied in the selection and prioritization of putative drug target genes; computational modeling and X-ray structure validation of protein targets with drug lead compounds; simulated docking and virtual screening of potential lead compounds; and lead validation and optimization using structure-activity and structure-function relationships. By identifying active sites, characterizing patterns of conserved residues and, where relevant, predicting catalytic residues, bioinformatics provides information to aid the design of selective and efficacious pharmacophores. The mathematical modeling study is required to develop the new antiviral drug, which may be used to control the disease outbreaks (Longini et al., 2005). The present study commences with prediction of 3-

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Received November 19, 2009; **Accepted** February 06, 2010; **Published** February 06, 2010

Citation: Singh S, Gupta SK, Nischal A, Khattri S, Nath R, et al. (2010) Comparative Modeling Study of the 3-D Structure of Small Delta Antigen Protein of Hepatitis Delta Virus. J Comput Sci Syst Biol 3: 001-004. doi:10.4172/jcsb.1000047

D structure of small delta antigen protein using comparative modeling and followed by evaluation of the predicted structure. The 3-D structure of small delta antigen protein is experimentally not known; therefore computational method was resorted to build a model through comparative modeling, allowing us to study its interactions. The evaluated 3-D structure was considered for further active site identification for docking studies. Till date, there is no effective drug available for delta hepatitis. The study outcome enables the identification of new lead molecule for targeting HDV.

Material and Methods

Sequence retrieval and alignment

The complete protein sequence of the target small delta antigen protein of HDV was retrieved from NCBI protein sequence database (Accession No- NP_077804.1), in FASTA format (http://www.ncbi.nlm.nih.gov/Proteins/). For identification of similar sequences BLAST (http://www. ncbi.nim.nih.gov/blast) (Altschul et al., 1990) database was used against the non-redundant protein sequences (nr) data. The BlastP was performed for homology search of structurally similar sequences with the protein data bank. GenThreader server was used for fold assignment. The alignment was done for target protein sequences with protein databank (PDB: 1A92) template using CLUSTALX (Thompson et al., 1997).

Prediction of 3-D structure via comparative modeling

The X-Ray diffraction structure of the oligomerization domain of hepatitis delta antigen of the Hepatitis delta virus was available (PDB: 1A92) and was used as template structure to generate 3-D model for small delta antigen protein. The X-ray 3-D structure of template was retrieved from http://www.rcsb.org/pdb/. The 3-D structure of target protein was generated by GenThreader (Jones et al., 1999) and Modeller9v7 (Sali and Blundell, 1993) tool using comparative modeling approach and visualization of 3-D structure was done by Swiss PdBviewer v 4.0.1.

Evaluation and validation of the 3-D structure

The Evaluation and validation of generated protein 3-D structure was done using software tools viz. PROCHECK and Anolea. PROCHECK (Laskowski et al., 2003) was used for validation of the 3-D structure of small delta antigen protein of HDV and energy minimization performed by Gromos96 (Christen et al., 2005) implemented via Swiss-pdb viewer (Guex et al., 2009). The overall stereochemical quality of the protein and the amino acid residues in the allowed, disallowed region and overall G-factor were assessed by Ramchandran plot analysis. The structural superimposition of Cα trace of the template and predicted structure of small delta antigen protein of HDV was performed by using Combinatorial Extension of polypeptides (http://cl.sdsc.edu/). The structures were visualized using Swiss PdBviewer v 4.0.1 and UCSF Chimera.

Active site prediction

Surface topography and ligand binding pockets of the predicted structure was performed using CASTp (Dundas et al., 2006). As determined by CASTp using a 1.4 Å radius probe, the internal cavity surface volume of the ligand binding sites was calculated.

Result and Discussion

The small form of hepatitis delta antigen (HDAg) functions as a trans activator of HDV replication cycle. The complete protein sequence of Small delta antigen protein (NP_077804.1) of Hepatitis delta virus was used in the study. The length of Small delta antigen protein is 195 amino acid, expected weight 21936.6 Da and isoelectric point (pI) was 10.02. The Protein BLAST for complete protein sequence of Small delta antigen protein was executed, hits was given <30% similarity with the target protein. Therefore, GenThreader was used to recognize the possible templates and fold assignment for 3-D structure prediction of small delta antigen protein of HDV (von Grotthuss et al., 2003). The outcome of GenThreader was given best similarity of small delta antigen protein with X-Ray diffraction structure of the oligomerization domain of hepatitis delta antigen (1A92A) of the Hepatitis delta virus (Table 1). The homology of Small delta antigen protein sequence showed 80% identity with the oligomerization domain of hepatitis delta antigen. Both the protein sequences of Small delta antigen protein of HDV and oligomerization domain of hepatitis delta antigen (1A92) was aligned and shown in Figure1.

Small delta antigen protein sequence was used to generate the 3-D structure using known X-ray 3-D structure 1A92A. Total 5 models were generated by Modeler9V7 and free energy of 3-D structures of Small delta antigen protein and template was evaluated, fifth one of them was considered to be thermodynamically stable and selected for further refinement and validation. The free energy of Small delta antigen protein of HDV was almost similar with the template.

The model was subjected to validation using PROCHECK and Anolea server. Ramachandarn plot shows that 91.5% residues in most favored region, 7.8% in additional allowed region and 0.7% in disallowed region. Consecutively, LYS106 residue of protein was subjected to loop refinement. Refine model was subjected to energy minimization using GROMOS96 implemented via Swiss-pdb viewer. The energy of final model was -7146.139 KJ/mol, which asses the thermodynamic stability of 3-D model. The stereochemistry of predicted structure was again evaluated. After energy minimization the Ramachandarn plot shows that 90.8% residues in most favored region, 9.2% in additional allowed re-

Conf.	Net Score	P-Value	PairE	SolvE	Aln Score	Aln Len	Str Len	Seq Len	Alignment	SCOP Codes
CERT	67.648	7e-06	-81.8	-5.8	329.6	50	50	195	1A92A0	h.4.6.1
MEDIUM	43.059	0.002	-28.9	-1.8	200.8	27	27	195	1BY0A0	h.4.6.1
LOW	30.124	0.046	-20.2	-6.5	104.0	35	363	195	1HV8A0	c.37.1.19
LOW	28.193	0.072	-56.4	-6.4	84.0	26	41	195	1HF9A0	h.4.8.1

[Conf = Description of confidence level, Score = Raw score from SVM, p-val = Probability of false positive, Epair = Pairwise energy for model, Esolv = Solvation energy for model, AlnSc = Sequence alignment score, Alen = Length of alignment, Dlen = Length of PDB entry, Tlen = Length of target sequence, PDB_ID = PDB identifier (+ chain code + domain code in CATH format)]

Table 1: Possible templates for 3-D structure prediction of small delta antigen protein of HDV identified by GenTHREADER.

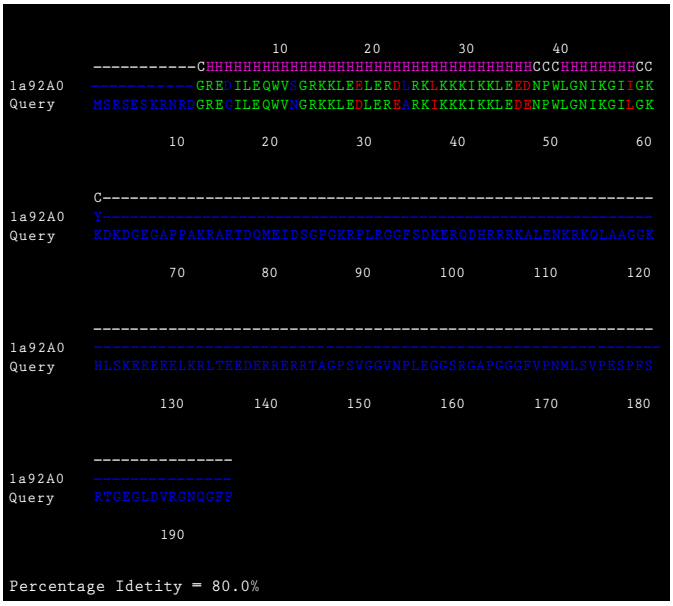

Figure 1: Sequence alignment of Small delta antigen protein of HDV and oligomerization domain of hepatitis delta antigen of HDV, showing helix and coils for 3-D structure prediction of small delta antigen protein of HDV by GenThreader.

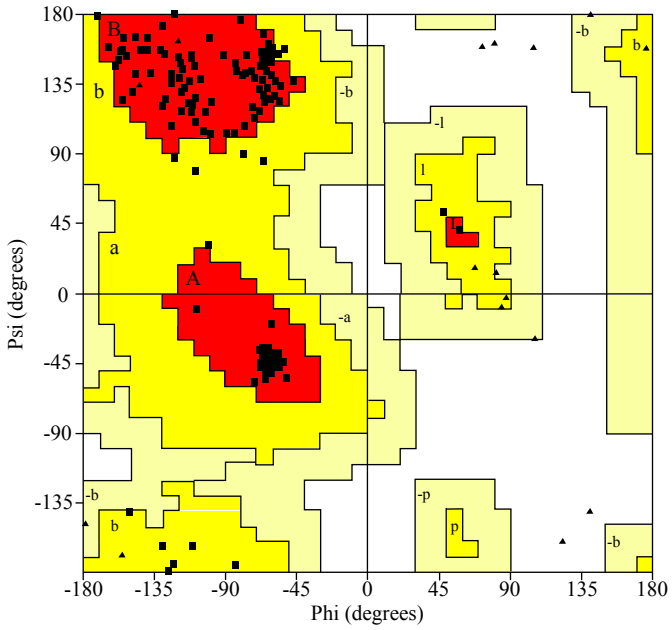

Figure 2: Ramachandran plot of the psi/phi distribution of the Small delta antigen protein model as obtained by PROCHECK: 90.8% residues are in most favored region and 9.2% are in additional allowed regions

gion and no residues are present in disallowed region (Figure 2). According to Ramachandran a good quality model should have >90% residues in favored region, thus the final model was validated as good quality model whose 3-D coordinates were viewed via Rasmol and UCSF Chimera tool (Figure 3) and it depicts alpha helix rich structure.

The quality of model was also assessed by comparing predicted structure to experimentally solved structure via superimposition and atoms root mean square deviation (RMSD) assessment. A model can be considered as reliable or accurate model when its RMSD is less than 3 – 4 Å (accurate <=2 Å reliable >=4 Å) (Rayan, 2009). Consequently, superimposition of the template oligomerization domain of hepatitis delta antigen of HDV with predicted structure of small delta antigen protein was executed by Combinatorial Extension of polypeptides. The weighted root mean square deviation of Cα trace between predicted structure of small delta antigen protein and template was 1.7 Angstroms with a significant Z-score 4.2; therefore it may be an accurate model.

CASTp server was used for Surface topography and functional binding site evaluation. In the final refine model 26 functional binding pockets were identified with probe radius as 1.4 Angstroms, among them 26th pocket was having largest area and conserved in both predicted model as well as in template. Thus in present study this site has been chosen as valid site for binding drug like molecule or inhibitor.

Conclusion

The present study was undertaken to model and validate the small delta antigen protein of HDV, which displayed several meaningful features: secondary structure, RMSD value, conserved residues engaged in non-bonded interaction. Study of surface topography for predicted 3-D model provided clue for interaction with inhibitor molecules to inhibit the virus replication.

Since the above study is in-silico, the predicted model can be useful to develop new inhibitor against HDV. The above study aims to serve all those researcher and pharmaceutical persons who are currently struggling for this incurable disease. The in-silico approach helps researchers by giving them an in-hand idea so that they can gladly advance towards the treatment of the disease.

Acknowledgement

The support of Department of Biotechnology, Ministry of Science and Technology, Government of India, to Bioinformatics Centre at Biotech Park Lucknow is gratefully acknowledged.

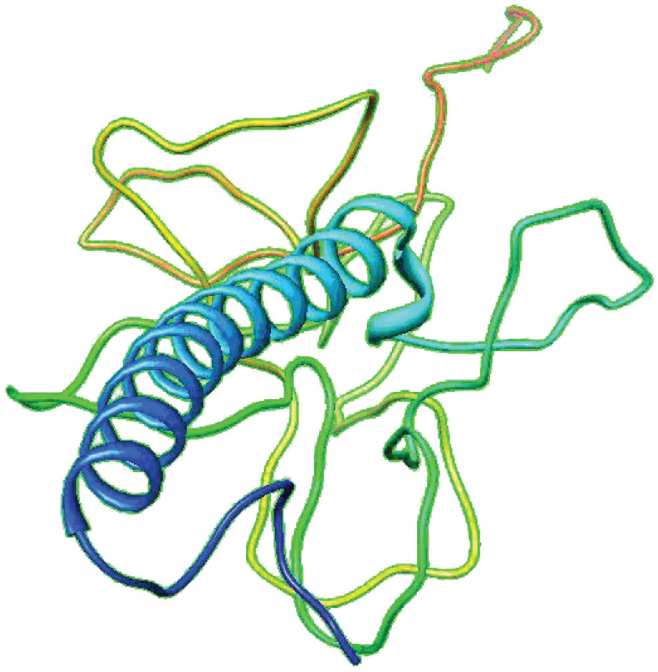

Figure 3: Final modeled 3-D structure of small delta antigen protein of HDV (Visualization in UCSF Chimera).

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