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Research Article

In silico Modeling and Drug Interaction Analysis of Molecular Structure of Ecto- Domain of E1 Glycoprotein of HCV - ③

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ABSTRACT

Over 2% world population has been estimated to be infected with Hepatitis C Virus (HCV) and it has been identified as a global threat for human health. In the current state of the art, there are some anti-HCV drugs which functions as inhibitors for viral RNA synthesis, however these drugs are associated with side effects which adversely affect the metabolic and physiological functions of the body. The envelope glycoprotein complex E1-E2 has been proposed to be essential for HCV entry into host cell. The efforts of developing vaccines against HCV are evolving; however there is no effective vaccine available for the management of HCV infection. As a part of developing candidate epitope vaccines against HCV, computational structural model of E2 glycoprotein ecto-domain has been proposed previously, but no advances were reported till date in case of E1 glycoprotein. In the present study we have attempted the modeling of E1 glycoprotein using *Ab initio* approach and deduced the structure using the *de novo* Rosetta fragment insertion method on ROSETTA Server. The models generated were evaluated using Procheck, WhatCheck/WhatIf. The promising model was used for docking studies using AutoDock, Docking server and SwissDock tools. Among the two selected anti-HCV drugs, Ribavirin (RBV) and Sofosbuvir (SFB), the docking studies revealed that RBV binding potential were lower than SFB, which infers that the RBV-glycoprotein E1 binding is comparatively stable than SFB. Moreover, the overall intermolecular energy with RBV binding was greater than SFB bound intermolecular energy. The results of the present studies may find applications in development of epitope vaccine targeting E1 ecto-domain glycoprotein of HCV.

Keywords: Hepatitis C virus; HCV infection; HCV glycoprotein; Molecular modeling; Molecular docking

INTRODUCTION

Hepatitis C is a viral disorder that brings about enlargement of liver and its infection is caused by the Hepatitis C Virus (HCV). It has been estimated that the global prevalence of HCV infection is around 2%, with 170 million persons chronically infected with the virus and 3 to 4 million persons newly infected each year [1,2]. The impact of this infection is just emerging in India [3]. Anti-viral therapy against HCV includes administration of Pegylated Interferon- α (PEG-IFN) with Ribavirin [4]. This antiviral therapy is not only very expensive but also shows toxic effects frequently [5]. Up until now, there is no vaccine available for HCV infection [6]. Hence, the development of effective, affordable therapeutic vaccines for HCV is very important in controlling chronic HCV infection. HCV possess single stranded positive sense RNA genome of around 9.5 kb that encode single poly protein precursor of 3011 to 3033 amino acids [7,8]. Upon protease activity, poly protein precursor yields two distinct types of proteins that can be categorized as structural (Core, E1, E2 and p7) and non-structural (NS2, NS3, NS4A, NS4B, NS5A and NS5B) proteins [9]. The fact that, viral envelope is formed by E1 and E2 glycoprotein heterodimers which are essential for virus entry into cells [10] makes E1 and E2 Glycoproteins an attractive target for vaccine design. Both the Envelope Glycoprotein E1 and E2 are type-I Transmembrane (TM) proteins, with N-terminal ecto-domains of 160 and 334 amino acids respectively, and a short C-terminal Transmembrane Domain (TMD) of approximately 30 amino acids. Structural information of these protein is very important not only in gaining insights into understanding the mechanism of entry of virus into human host, but also in design and development of effective peptide vaccines against HCV. Computational structural model of E2 glycoprotein ecto-domain had been proposed [11], since then no advances are reported till date in case of E1 Glycoprotein.

Among the conventionally used therapeutic molecule against HCV virus, we have selected Ribavirin (RBV; PubChem CID- 37542) [12] and Sofosbuvir [13] for studying the molecular interactions with E1 glycoprotein model of HCV. RBV is a synthetic nucleoside analog of ribofuranose with activity against hepatitis C virus and other RNA viruses. RBV is incorporated into viral RNA, thereby inhibiting viral RNA synthesis, inducing viral genome mutations, and inhibiting normal viral replication. Currently it is prescribed as therapeutic modality against both RNA and DNA viruses.

Sofosbuvir (SFB; PSI-7977) is a uridine monophosphate analog inhibitor of HCV NS5B polymerase and is currently prescribed as antiviral agent in the management of chronic HCV infections. The mechanism of action of Sofosbuvir is as a RNA replicase inhibitor. Upon oral administration, SFB is metabolized to 2'-deoxy-2'-alpha-fluoro-beta-C-methyluridine-5'-monophosphate, which is then converted into the active triphosphate nucleotide that inhibits the NS5B polymerase, thereby preventing viral replication.

In the present investigations, attempts have been made to develop the structural model of E1 glycoprotein of HCV using *Ab initio* modeling approach and the utility of the developed model towards its drug binding potential was performed using molecular docking studies.

METHODS

Sequence retrieval, elementary analysis and model generation of E1 glycoprotein

Entire Genome Polyprotein of HCV was downloaded from SWISSPROT (<http://www.uniprot.org>) [14] with Swissprot id P27958. Initially a BLAST search [15] was performed against PDB database [16] from NCBI interface (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to find any homologous sequence that can be used as template in homology modeling. In outcome from BLAST, none of the available structure showed significant sequence similarity with E1 Glycoprotein. Alternatively, fold recognition method was employed from PHYRE Server (<http://www.sbg.bio.ic.ac.uk/~phyre/>) [17] to identify conserved fold in the sequence. In result, none of the fold recognized can be used for model building. Finally, considering the small size (159 amino acid residues) of ecto-domain of E1 Glycoprotein, *Ab initio* approach was utilized to deduce the structure using the *de novo* Rosetta fragment insertion method on ROSETTA Server (<http://rosetta.bakerlab.org/>) [18].

Model evaluation, refinement, energy minimization and visualization

Five best models obtained from Robetta server were subjected to stereochemical checks using Procheck server [19] from Swiss model interface (<http://swissmodel.expasy.org>) [20] and WhatIf [21] web server (<http://swift.cmbi.ru.nl/servers/html/index.html>) was utilized to analyze protein on various factors like inter-atomic bumps, abnormal packing environment, side chain flips and other 78

such factors that reflects the chemical correctness of protein. Model refinement was carried out for removal of interatomic bumps by rotating side chain torsion angles using WhatIf Web Server. Energy minimization was performed using Force field approach by means of GROMOS96 43Bl parameters set [22], without reaction field applying 40 steps of Steepest Descent Algorithm followed by 20 Steps of Conjugate Gradient algorithm within DEEP VIEW 3.7 [23]. Models were visualized using The PyMOL Molecular Graphics System and can be retrieved from PMDB Database with PMDB ID PM0077558 [24].

Docking studies

Target structural model of HCV E1 glycoprotein was used for molecular docking studies. Before proceeding to actual virtual screening, the coordinate file was edited appropriately to remove non protein parts with the help of Discovery Studio 3.5 for preparing receptor [25]. The target system was generated in conventional .pdb format. 3D structure of RBV and SFB were obtained from PubChem and saved in .pdb format using Discovery Studio 3.5. Docking calculations were carried out using DockingServer (www.dockingserver.com) [26]. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged and rotatable bonds were defined. AutoDock tools were used for adding essential hydrogen atoms and for addition of necessary hydrogen atoms and solvation parameters to the target protein structure [27]. Affinity (grid) maps of $40 \times 40 \times 40$ Å (x, y and z) grid points and 0.375 Å spacing were automatically generated using the AutoGrid program [27]. AutoDock parameter set and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. The MMFF94 force field was used for the energy minimization of ligand molecules (RBV and SFB) using Docking server [28]. Docking simulations were performed using the Lamarckian Genetic Algorithm (LGA) and the solis and wets local search method [29]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å and quaternion and torsion steps of 5 were applied.

RESULTS AND DISCUSSION

Sequence retrieval, elementary analysis and model generation of E1 glycoprotein

BLAST results comprised of 8 hits, of which only the very first hit had acceptable E-value ($8e^{-14}$), remaining hits showed E value greater than zero hence cannot be relied on. The first hit obtained had very poor query coverage (only 29 residues were found to be aligned) that spanned the transmembrane region and not the ecto-domain. The results obtained from BLAST clarifies the fact that homologous structure do not exist that can be used for homology modeling, therefore another approach of fold recognition needs to be applied. PHYRE Fold recognition server predicted 10 putative folds. Only first fold had appropriate percentage of precision (90%) but it also had very poor query coverage (only 21 amino acid residues from query had been modelled in fold). The remaining folds either showed poor precision percentage or had very less query coverage. Finally, Robetta *Ab initio* structure prediction server build 5 models based on identified PFAM parent fold PF01539 from Glnzu prediction. Models obtained from Robetta server had estimable confidence score of 93.53 and span entire query length (Figure 1).

Model evaluation and refinement

The results of Procheck analysis have been summarized in figure 2. It is clear that the five models generated from Robetta server performed well on stereo chemical checks. Model 2 seems to be best owing to its 97.8% residue in core region, 2.2% in additional allowed region and none of residue in generously allowed or disallowed region of the Ramachandran plot. Both model 3 and 4 have 96.4% of its residue in core region, 3.6% in additional allowed region and none of residue in generously allowed or disallowed region of the Ramachandran plot. Although model 5 has 94.9% of its residue in core region as compared to 95.7% in Model 1, but comparatively model 1 is better than model 5 as it has got less percentage of residues in additional allowed (2.9% for model 1 and 5.1% for model 5), generously allowed (0.7% for model 1 and none of residue from model 5) and disallowed region (0.7% for model 1 and none of residue from model 5). The results of the WhatIf/WhatCheck analysis have been summarized in figure 3. The results obtained shows that all the models achieved good results in WhatIf/WhatCheck analysis (Figure 3), except some interatomic bumps were observed in all the models. Model 3 and 5 showed two and one side chain flips respectively, remaining models passed clean on this standard. Bumps were observed maximum in model 5 (198) followed by model 4 and 1. Model three reported 110 clashes and model 2 possess lowest number of bumps (105). In view of the results from both Procheck and Whatif/Whatcheck (Figure 3), it is clear that the model 2 stands best on all structural tests. Hence model 2 was considered further for molecular docking studies.

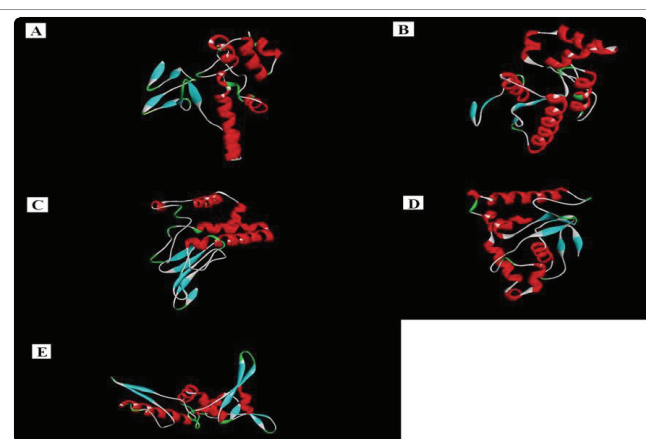


Figure 1: Structural view of five different models of glycoprotein E1 domain of HCV obtained from Robetta server.

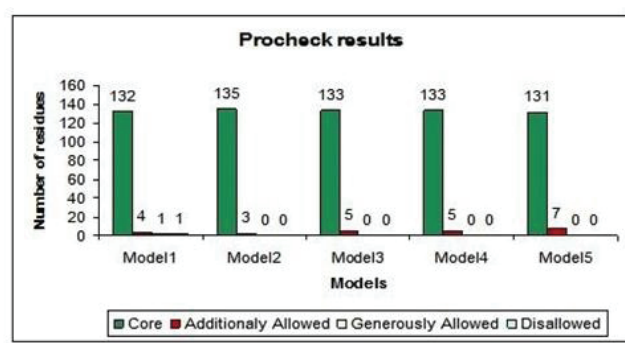


Figure 2: Profile of PROCHECK analysis of the five E1 ecto-domain models of HCV generated from Robetta Server.



Despite of significant progress made in the computational protein modeling, the further quest of improving the quality of models is still evolving. There are several limitations and shortcomings in this regard, for example models often represent only fractions of the full-length of desired protein leaving behind the unresolved questions in template-based modeling to combine information from multiple templates such as different structural domains, into larger complex assemblies. The development of consistent, accurate and progressive methods for improvement of models by shifting the coordinates parallel to the native state is one of the evolving research area in the protein modeling.

Molecular docking

Molecular docking studies were carried out using Docking server, wherein the parameters of free energy of binding, inhibition constant (Ki), total estimated energy of vdW + Hbond + desolv (E_{VHD}), electrostatic energy, total intermolecular energy, frequency of binding, and interact surface area were calculated to determine the favourable binding of ligand molecules with the target protein. The molecular docking of RBV and SFB with E1 glycoprotein (Model 2) using Swiss DockServer resulted in 49 and 47 clusters respectively. The top-score cluster had significantly lower full fitness [30] than the others. This binding site is formed by residues at the C-terminal end of each subunit in the E1 ecto-domain, close to residue Phe 94 and Gly 97. In this conformation, RBV could be able to form three hydrogen bonds with the backbone amide of Ser 77, Gly 97 and Thr 101, making it a favourable binding site (Figure 4-7). However, the binding analysis of SFB revealed the interactions with two polar molecules, Arg 105 and His 121. Thus, binding of RBV and SFB to their respective adjacent sites might lead to a greater packing and conformational stability of this part of the protein (Figure 4-7).

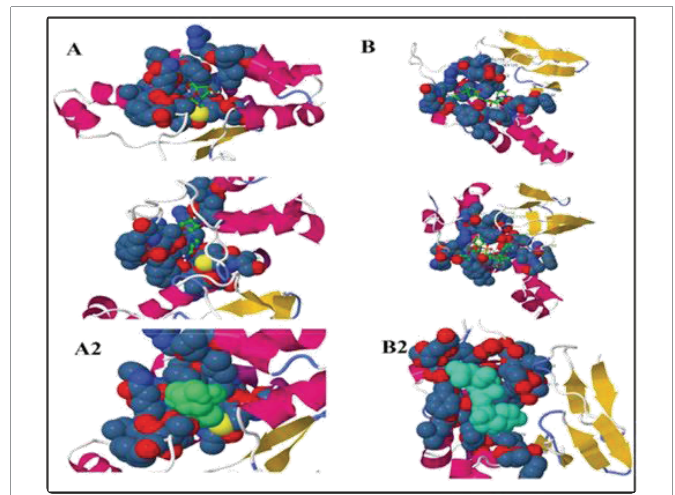


Figure 5: Visualization of RBV/SFB-E1 ecto-domain glycoprotein interaction profile using Docking server. (A) RBV-E1 Glycoprotein complex: HCV glycoprotein E1domain cartoon interacting with RBV (ball and stick) (A2) Surface view of RBV-E1 domain complex: RBV (green surface) interacting with side chains of E1 domain (blue and red surface). (B) SFB-E1 domain complex: HCV glycoprotein E1domain cartoon interacting with SFB (ball and stick). (B2) Surface view of SFB-E1 domain complex: SFB (green surface) interacting with side chains of HCV Glycoprotein E1 ecto-domain (blue and red surface).

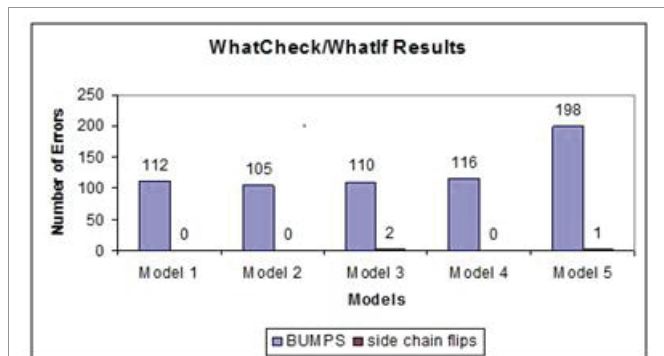


Figure 3: Summary of Whatif/WhatCheck analysis of the E1 ecto-domain models of HCV.

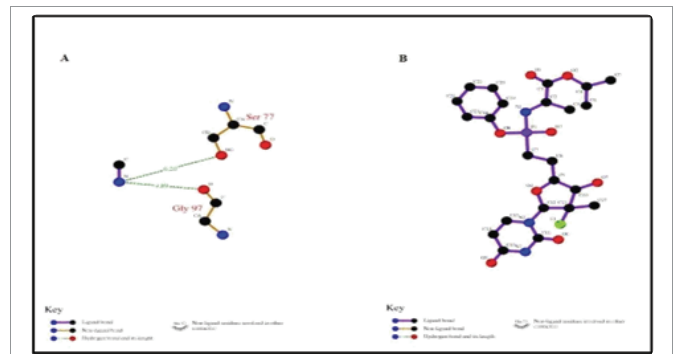


Figure 6: The 2D plot of interaction of RBV/SFB with E1 ecto-domain of HCV generated using Docking server. (A) 2D plot of RBV-E1domain interaction. (B) 2D plot of SFB-E1 domain interaction. The legends for ligand bond, non-ligand bond, hydrogen bonds and its length are mentioned.



Figure 4: Docked complexes of RBV (A) and SFB (B) with E1 glycoprotein ecto-domain of HCV.

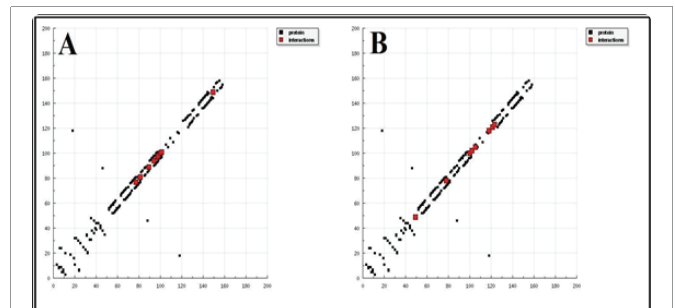


Figure 7: The HB plot of RBV/SFB-E1 ecto-domain protein interaction profile generated using Docking server. (A) The HB plot structure of RBV-E1 ecto-domain interaction. The amino acid residues involved in interactions were observed to be 77: Ser, 81: Cys, 89: Leu, 94: Phe, 97: Gly, 98: Gln, 100: Phe, 101: Thr, and 149: Ile. (B) The HB plot structure of SFB-E1 ecto-domain interaction. The amino acid residues involved in interactions were observed to be 49: Val, 78: Ala, 100: Phe, 102: Phe, 105: Arg, 118: Tyr, 121: His and 123: Thr.


Table 1: The docking interaction parameters of both RBV and Sofosbuvir with E1 glycoprotein model of HCV.

Ligands	Binding free energy (Kcal/mol)	Total Intermolecular energy (Kcal/mol)	Interacting amino acids	Hydrogen bonds	Residues in hydrophobic interaction
RBV	-4.35	-4.03	SER77, CYS81, LEU89, PHE94, GLN98, PHE100, THR101, ILE149, GLY97	N - SER77[3.28]; N- GLY97[3.40]; N -THR101[3.19]	C - CYS81[3.62]; C5* - PHE94 [3.16]
Sofosbuvir	-3.48	-7.07	VAL49, ALA78, PHE100, PHE102, ARG105, TYR118, HIS121, THR123	-	C6 - PHE10 [3.63]

The residues involved in the binding interactions of RBV and SFB are summarized in table 1. The docking results shows that the estimated free energy of binding was of -4.35 kcal/mol, for the most favourable binding pose of RBV and total intermolecular energy was of -4.03 kcal/mol. In case of binding of SFB, estimated free energy of binding was of -3.48 kcal/mol, and total intermolecular energy was of -7.07 kcal/mol. As compared to SFB, RBV exhibited comparatively low free energy of interaction and intermolecular energy. In order to study the interactions of protein-ligand, a 2D plot was generated where ligand bond, non-ligand bond, and hydrogen bonds along with their length were mentioned (Figure 6). HB (hydrogen bonding) plot is a novel tool being used for unravelling protein structure and function by describing structure in concert with network of hydrogen bonding interaction (Bikadi et al). A HB plot was generated to analyze the interactions of RBV and SFB with different amino acids of the E1 glycoprotein domain of HCV (Figure 7). The HB plot structure of RBV-E1 ecto-domain interaction revealed the involvement of 77: Ser, 81: Cys, 89: Leu, 94: Phe, 97: Gly, 98: Gln, 100: Phe, 101: Thr, and 149: Ile in interaction with RBV. While the HB plot analysis of SFB-E1 ecto-domain revealed the involvement of 49: Val, 78: Ala, 100: Phe, 102: Phe, 105: Arg, 118: Tyr, 121: His and 123: Thr in interaction with SFB (Figure 7). Although the docking experiments has significantly contributed towards the process of drug discovery, however the accuracy and speed of docking calculation is a challenge which limits the authenticity of obtained results.

CONCLUSION

In the present investigation, by employing an *Ab initio in silico* approach we have attempted to develop and validate a structural model of E1-ecto domain glycoprotein of HCV using the *de novo* Rosetta fragment insertion method on ROBETTA Server. Based on Procheck Ramachandran Plot analysis, among 118 structures of resolution of at least 2 Å and R-factor no greater than 20%, a bunch of 5 good quality models for HCV glycoprotein E1 were observed to have over 90% residues in the most favoured regions. Therefore these models in general and model 2 in specific can be further explored for design and development of anti-HCV epitope vaccines targeting E1 ecto-domain which per say is involved in entry of HCV within the human cell. While investigating the utility of developed models, the docking studies revealed that RBV (a Drug against HCV) interacted with more number of residues as compared to SFB molecule. Nevertheless, RBV-E1 ecto-domain complex is highly stable owing to presence of hydrogen bonding, whereas SFB-E1 ecto-domain showed lower stability owing to lack of hydrogen bonding. Owing to the non-availability of the effective small molecule drug or vaccine drug candidate for the management of HCV, the results of the present investigation might serve as a ready reference for design and development of novel anti-HCV epitope vaccine/s targeting E1-ecto-domain of HCV.

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