Designing and molecular docking of cyclic peptides against HCV NS3 protease

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Abstract: Hepatitis C Virus (HCV) infection is a worldwide serious health issue which contributes towards most of the hepatic morbidities. So far no prophylaxis is available to prevent this virus; therefore, development of antiviral compounds to fight HCV infection is the need of time. Chemically synthesized peptides that are potent immunogenic antigens are being pursued as candidate vaccines against HCV. The present study was planned to identify peptide inhibitors having potential to block the activity of NS3 protein of HCV that will ultimately arrest HCV multiplication. Docking of NS3 with peptides revealed that the majority of the peptides have strong binding affinity for active sites of NS3. Peptide 1, 2, 3 and 6 were found interactive with NS3 active residues while the active sites of NS3 had hydrophobic contact with the rest of peptides. Thus, these peptides bear therapeutic potential of a candidate drug for the prevention of HCV replication. Post docking analysis revealed important binding abilities of peptides with the active sites of NS3 protein, showing the efficiency of peptides as potential peptide inhibitors against HCV. The study revealed that HCV replication can be inhibited by these peptides. HCV replication inhibition potential of these peptides can contribute in reducing the burden of HCV infection and its associated complications worldwide.

Keywords: HCV NS3 protease, peptide inhibitors, interferon, docking.

INTRODUCTION

Hepatitis C virus (HCV) holds a prime position in the etiology of liver diseases affecting around 200 million people globally, with the highest prevalence in African and Asian countries. High rate of incidence and severe morbid conditions associated with persistent HCV infection have made it a deadly disease. (Ashfaq, Khan *et al*., 2011, Bostan and Mahmood, 2010). HCV can cause cirrhosis and hepatocellular carcinoma (Jahan, Ashfaq *et al*., 2012). HCV belongs to *Flaviviradae* family and its genome is a single stranded RNA molecule. It replicates very rapidly as the proportion of HCV infected hepatocytes ranges between 5-100%. The HCV genome encodes 10 viral proteins Core-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-COOH. All these ten proteins play an important role in HCV pathogenesis and are crucial for entry, multiplication and other cellular processes of the pathogen (Ashfaq, Yousaf *et al*., 2011, Idrees and Ashfaq, 2013). HCV has six genotypes and more than 50 subtypes. A genotype with a novel sequence has been identified and named as subtype 7a (Nakano, Lau *et al*., 2012). Among all genotypes, genotype 3a is most likely responsible for liver fibrosis (Rubbia-Brandt, Fabris *et al*., 2004, Westin, Nordlinder *et al*., 2002). Because of high strain variation, there is no vaccination available to combat HCV infection. At present, the recommended therapeutic regimen for HCV is pegylated alpha interferon (IFN-α) either alone or in combination with ribavirin (Idrees and Ashfaq, 2013). This treatment is less efficient, costly and is associated with many side effects. Thus, development of antiviral compounds, which can efficiently fight against HCV infection and are effective against all genotypes with least side effects, is an urgent and dire need of time. (Ashfaq, Javed *et al*., 2011, Ashfaq, Masoud *et al*., 2011).

The nonstructural protein (NS3) is a 67 kDa multifunctional protein that has serine protease domain at the N - terminal end and an NTPase/helicase domain at the C - terminal (Gallinari, Brennan *et al*., 1998). A significant research has focused on protease activity of NS3 as NS3 targets a Mitochondrial Antiviral Signaling protein (MAVS) which is responsible for NF- NF-κB and IFN regulatory factor 3 activation (Li, Sun *et al*., 2005). NS3 protein due to its prime role in HCV replication has become an important drug target to find potential cures for HCV (Idrees and Ashfaq, 2013). Potent immunogenic chemically synthesized peptides are being developed as vaccine candidates for HCV (Lechmann and Liang, 2000). Therefore, this study was designed to identify molecules having peptide inhibition activity against HCV NS3 protein which sequentially can lead towards HCV replication arrest and can reduce burden of HCV and its associated complications on world population.

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MATERIALS AND METHODS

This work was intended to discover biomolecules effective for peptide inhibition against HCV NS3/4A protein by performing molecular docking using the MOE (Molecular Operating Environment) software package.

Receptor protein optimization

Three-dimensional (3D) structure of the HCV NS3/4A protease was retrieved from the database of Protein Data Bank (PDB) using PDB ID: 3P8N. It was an X-RAY DIFFRACTION structure with resolution of 1.90 Å. Already bound inhibitor was removed from NS3/4A complex and structure was optimized by using Molecular Operating Environment (MOE) (MOE, 2012). Water molecules were eliminated for the optimization of construct and protonation was performed to alter the condition into ionization level. Furthermore, energy of the structure was minimized considering Force Field: MMFF94X+Solvation, gradient: 0.05 and Chiral Constraint: Current Geometry. Docking and simulation analyses were performed on reduced structure.

Devising peptide

The particular site where peptide binds on the HCV NS3 becomes selective for substrate residues containing amino acids with positive charge. One study revealed that the P1 position of the NS3's binding site has a high selectivity for alkaline amino acid residues (Arg/Lys), whereas the preference for the P2 is Arg > Thr > Gln/Asn/Lys and for P3 is Lys > Arg > Asn**.** Keeping this in mind, penta-peptides and hexa-peptides consisting of combinations of these amino acids were designed. A total of 10 Peptides (5 penta and 5 hexa) were designed that could serve as inhibitors against HCV NS3/4A protease. Cysteine residues were added on both sides of peptides to form a disulfide bridge so that cyclic peptides can be designed. All the premeditated cyclic peptides were transformed to relevant 3D structures through Chem Sketch software (ACD/ChemSketch, 2012).

Optimization of the peptides

3D structures of peptides were optimized by minimizing energy through MOE software. The parameters used for peptide energy minimization were gradient: 0.05, Force Field: MMFF94X, Chiral Constrzaint and Current Geometry. Library of peptides was maintained in mdb format for its further use in MOE-Docking as input file.

Molecular docking

Docking of peptides with active sites (His 57, Asp 81, Ser 139) of receptor protein was accomplished through MOE docking program by using parameters like placement: triangle matcher, Re-scoring function: London dG, Retain: 10, Refinement: Force field, and Re-scoring 2: London dG. Best conformations of docking on the basis of the lowest S -score were analyzed for H- bonding/π-π interactions. Chimera Molecular Structure visualization software was used to take high quality images of interactions among peptides and NS3/4A protein.

RESULTS

Crystal structure of NS3/4A protein was retrieved using PDB ID: 3P8N. This structure was selected as it was having resolution of 1.90 Å. All ligands were docked with the catalytic triad of NS3/4A complex (fig. 1).

Molecular docking

After docking, S- score values were used as a parameter to store all conformations. The best conformations were subjected to further analysis. Peptide 6 was graded as the most excellent conformation followed by Peptide 3, 5, 4, 2 and 7. S-score values of the rest of conformations were near most equal (table 1). Best conformation for every peptide was investigated to determine the dynamics of interaction. Interactions were further checked through LigPlot+ and Chimera visualization software.

Interaction analysis

Conformations with the lowest E score were selected as the best ones and were analyzed for H-bonding. Peptide 1 was having three atomic interactions. First interaction was between ligand atom N3 and receptor atom O of Asp 79 with a bond distance of 3.29. N6 was interacting with OD2 of Asp 81 with a bond distance of 3.05. O6 was interacting with NZ of Lys 136 with a bond distance of 2.86. Peptide 2 had six atomic interactions with NS3 residues. N3 atom of ligand had interaction with O of Val 78 and O of Asp 79 with a bond distance of 3.27 and 2.98 respectively. O1 atom of ligand had interaction with NE of Arg 155 with a bond distance of 3.29. N10 of ligand had interactions with OE1 of Gln 41 and OG of Ser 139 with a bond distance of 3.16 and 3.05 respectively. N9 had interactions with OG of Ser 139 with a bond distance of 3.31. Peptide 3 had two hydrogen bonds with receptor protein. N7 atom interacted with OD2 of Asp 81 with a bond distance of 2.98 and O6 of ligand had a hydrogen bond of 2.93 with atom NH2 of Arg 123. Peptide 4 had two hydrogen bonds with receptor protein. Atom N6 interacted with O of Val 78 with bond distance of 3.29. N1 interacted with O of Asp 79 and bond distance was 3.10. Peptide 5 formed six hydrogen bonds with receptor protein. N3 of peptide interacted with O of Asp 79 with a bond distance of 2.88. N4 interacted with O of Asp 79 with a bond distance of 3.06. O1 interacted with NE of Arg 155 and bond distance was 3.09. O6 interacted with NZ of Lys 136 with a bond distance of 2.92. N8 interacted with O of Alan 157 with a bond distance of 2.98. N11 had interactions with OE1 of Gln 41 with a bond distance of 2.76. Peptide 6 formed four hydrogen bonds with receptor protein. N1 interacted with O of Asp 79 through hydrogen bond (bond length: 2.88). N8 formed hydrogen bonds (bond length: 3.09) with OD2 of Asp 81. N11 formed

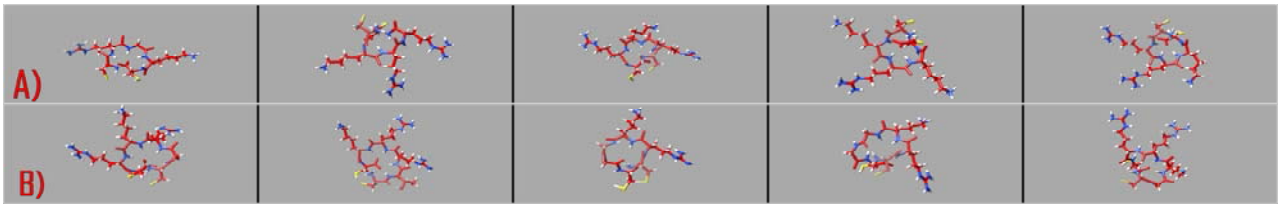

Fig. 1: 3D structures of cyclic peptides. A. 3D structures of penta peptides. B. 3D structures of hexa peptides.

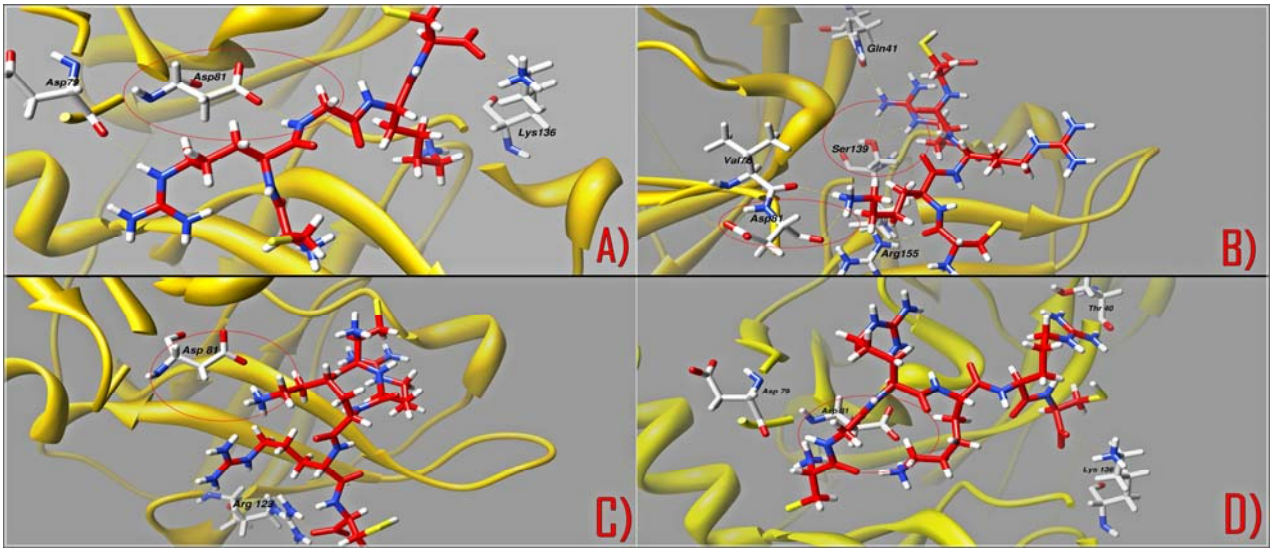

Fig. 2: Binding interactions of peptide 1,2,3 and 6 with the active sites of NS3/4A. A. Interaction of peptide 1 with active site Asp 81. B. Interaction of peptide 2 with active site Asp 81 and Ser 139. C. Interaction of peptide 3 with active site Asp 81. D. Interaction of peptide 6 with active site Asp 81.

hydrogen bonds (bond length: 3.11) with O of Thor 40. O7 interacted with NZ of Lys 136 and bond length was 2.89. Peptide 7 formed two hydrogen bonds with receptor protein. N5 formed hydrogen bonds with O Ala 157 with a bond distance of 3.15. O8 interacted with NZ of Lys 136 with a bond distance of 2.87. Peptide 8 had two hydrogen bonds with receptor protein. O5 had a hydrogen bond (bond distance: 3.05) with an N of Gly 137. O7 had interaction with O of Leu 135 with a bond distance of 3.23. Peptide 9 had five interactions with receptor protein. O1 interacted with NZ of Lys 136 with a bond distance of 3.28. O4 interacted with NE2 of Gln 41 with a bond distance of 3.08. O5 and O7 interacted with NZ of Lys 62 with a bond distance of 3.29 and 3.09 respectively. O6 had interaction with N of Ser 61 with a bond distance of 3.12. Peptide 10 had three interactions with receptor protein. N1 interacted with OG1 and O of Gly 58 with a bond distance of 3.09 and 2.95 respectively. O7 interacted with NZ of Lys 136 with a bond distance of 2.79. Among all the peptides, peptide 1, 2, 3 and 6 had hydrogen bonding with two active sites namely Asp 81 and Ser 139 while all other peptides had no hydrogen bond with any catalytic site. All peptides had hydrophobic contact with a catalytic triad of NS3 protease and among all peptides peptide 4 had hydrophobic contact with all three catalytic sites. The findings of the study lead to the conclusion that these peptides can potentially act as inhibitors of the HCV NS3 protein. Interacting residues of the receptor protein are shown in table 1. Interactions between receptor and ligands are shown in fig. 2.

DISCUSSION

Hepatitis C is a severe morbid condition and development of successful therapies that can efficiently stop HCV infection are badly required. Currently, there is no vaccination available to prevent this disease and the approved treatment being used is a combined use of ribavirin and pegylated interferon α (Idrees and Ashfaq, 2013). Non- structural protein NS3 is constituted by serine protease and helicase at N and C terminal respectively. In recent years, NS3 protease has become an attractive target to develop antiviral compounds whereas tremendous efforts are being done to target helicase domain and design novel inhibitory compounds. The mitochondrial antiviral signaling protein, MAVS is targeted by NS3 therefore, NS3 has become an attractive drug target in the war against HCV infection (Li, Sun *et al*., 2005, Pang, Jankowsky *et al*., 2002). To date many NS3 inhibitors have been screened and recently, two of them have been approved by FDA (Li, Brecher *et al*., 2017, Matthew, Zephyr *et al*., 2017). Recently reported

Table 1: Peptide interactions with HCV NS3/4A protein. Active sites are highlighted in red.

Sr. No.	Peptide	E Score (S)	Interacting Residues (Hydrogen bonding)	Hydrophobic Contact Residues
1	CRGKC	-12.66128	Asp 79, Asp 81, Lys 136	Asp 168, Arg 155, Ala 156, Phe 154, His 57
2	CKRRC	-13.21904	Val 78, Asp 79, Arg 155, Gln 41, Ser 139	Asp 168, Arg 123, Asp 81, Ala 156, His 57, Lys 136
3	CRKRC	-13.40228	Asp 81, Arg 123	His 57, Leu 135, Lys 136, Ala 157, Ala 156, Val 158, Arg 155, Asp 168
4	CKRKC	-13.33338	Val 78, Asp 79	Asp 168, Arg 155, Arg 123, Ala 157, Phe 154, Ala 156, Ser 139, Lys 136, Gln 41, His 57, Asp 81
5	CRRKC	-13.36615	Asp 79, Arg 155, Ala 157, Gln 41, Lys 136	Asp 81, Asp 168, Arg 123, Gly 137, His 57, Ala 156, Val 132, Leu 135
6	CGRKRC	-13.55317	Asp 79, Asp 81, Thr 40, Lys 136	Gln 80, Arg 155, His 57, Gln 41, Tyr 56
7	CTRRKC	-13.07805	Ala 157, Lys 136	Gln 80, Asp 79, Ser 122, Arg 155, Val 158, Ala 156, His 57, Val 78, Tyr56, Asp 81
8	CRNKKC	-7.288572	Gly 137, Leu 135	His 57, Lys 136, Phe 154, Ser 139, Ala 156
9	CRKGGC	-11.38387	Lys 136, Gln 41, Lys 62, Ser 61	Ala 156, Phe 154, His 57, Gly 58, Gly 60
10	CGKRRC	-11.5564	Gly 58, Lys 136	Lys 62, Gly 60, His 57, Arg 155, Ser 139, Asp 81

crystal structure of NS3 has opened new vistas to develop novel and efficient drug compounds by providing knowledge of NS3 domains and their affinity to bind with drug candidates.

Bioinformatics and computational biology have enabled researchers to check the affinity of drug candidates against key viral genes and have significant contribution in the development of new antiviral compounds by providing the knowledge of molecular interactions among inhibitors and target proteins. An oxyanion hole and a catalytic triad (His-57, Asp-81 and Ser-139) is needed for the activity of NS3 protease. Proper positioning of the catalytic triad and the substrate is maintained by NS4A cofactor, therefore NS4A cofactor is important in substrate specificity and catalytic efficiency (Raney, Sharma *et al*., 2010). Since catalytic triad is an integral component of HCV replication therefore, its targeting possibly will cease the replication of HCV and reduce the threat of HCV infection.

The current study was designed to check the replication inhibition potential of penta and hexa peptides by their molecular docking against NS3 protease. Molecular docking is a computational technique to find the best fit orientation ligands with proteins of interest. Therefore, molecular docking is believed as a useful technique to screen novel inhibitory compounds/therapeutic agents against deadly diseases (Lengauer and Rarey, 1996). During this study, potential of peptides (penta and hexa) as HCV replication inhibitors was determined by their docking against NS3 protease. After docking, only the most excellent conformations were chosen and were subjected to interactions analysis. Peptide 1,2,3 and 6 were found potentially interacting and capable of successfully blocking NS3 protease. The results support to conclude that these peptides are key molecules for drug development as these peptides can successfully block HCV replication. Though, interactions among designed peptides and NS3 protease have been identified and characterized, yet these results need validation through *in-vitro* and *in-vivo* studies to check their potential as HCV replication inhibitors.

CONCLUSION

Molecular docking of NS3/4A and peptides was carried out to identify peptides capable of NS3 protease inhibition. This study has revealed potential binding interactions of peptides with the active sites of NS3 protease. The knowledge of binding interactions of these peptides can be helpful before *in-vitro* and *in-vivo* pharmacological evaluation to save time and cost. These observations in this study suggest that these peptides have binding affinity against NS3 protease and can play as principal inhibitors against NS3/4A protease of HCV.

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