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Full Length Research Paper

Computational studies on Ribavirin binding to RNA-dependent RNA polymerase, inducing Crimean-Congo hemorrhagic fever

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The Crimean-Congo hemorrhagic fever is a viral disease, widespread amongst domestic as well as wild animals, and probably may affects humans too. This virus belonging to Bunyaviridae family contains viral RNA-dependent RNA polymerase catalyzing the biosynthesis of the RNA strands. As a consequence of inhibition of a target enzyme protein, namely, RNA-dependent RNA polymerase there is a probability of the prevention of virus replication in the host cells. In the present investigation, RNA-dependent RNA polymerase sequence was retrieved from NCBI followed by its domain analysis to find out the effective part of the sequence later on 3D structure of the domain modeling by comparative homology modeling approach, including a thorough screening of the homologous sequences to domain by sequence alignment technique. Template coordinates are then used as outline for modeling the domain structure. The computed models of RNA-dependent RNA polymerase domain was validated and then optimized by employing Ramachandran plot. Homology models of RNA-dependent RNA polymerase domain was finally taken into consideration to focus on the prediction of the specific ligand binding site by docking against antiviral drug Ribavirin, with an aid of AutoDock4.2. The results obtained from the present studies provide new insights into the broad screening of certain putative marker inhibitors of the RNA-dependent RNA polymerase concomitant with their application in the relevant drug designing.

Key words: Crimean-Congo hemorrhagic fever virus, homology modeling, Ribavirin, RNA-dependent RNA polymerase.

INTRODUCTION

The Crimean-Congo hemorrhagic fever virus (CCHFV) is from the family Bunyaviridae, and causes severe disease in animals and humans too, with reported mortality rates up to approximately 80% (Ergonul et al., 2004). This virus contains circular RNA as genetic material. RNA fragments therein are recognized as the small (S), the medium (M) and the large (L) fragments, according to their size (Clerx et al., 1981; Wishart et al., 2006). As a consequence, the S-fragment constitutes the smallest portion and encoded the structural nucleocapsid proteinaceous component. It acts to protect the RNA and, concomitant with the viral polymerase, lead to coordinate with the viral transcription and replication. The glycoproteins are remarkably encoded by the M-fragment. These putative surface enzymatic proteins project through the viral lipid envelope and are thus solely accountable for viral anchoring and door- way to the host cell (Alvarez et al., 2006). The L-fragment encodes the viral RNA-dependent RNA polymerase and is recognized for uniqueness due to its nearly two fold larger size compared to that of L-fragment of other members of Bunyaviridae family (Honig et al., 2004). In 1950s, a virus with similar pathogenesis was isolated in 1956 from patient in Congo, Africa and the virus was subsequently named as Crimean-Congo hemorrhagic fever (CCHF) virus (Hoogstral, 1979; Laskowski et al., 1993), with the geographic occurrence in sub-Saharan Africa, Bulgaria, northern Greece, Soviet Union (particularly the former

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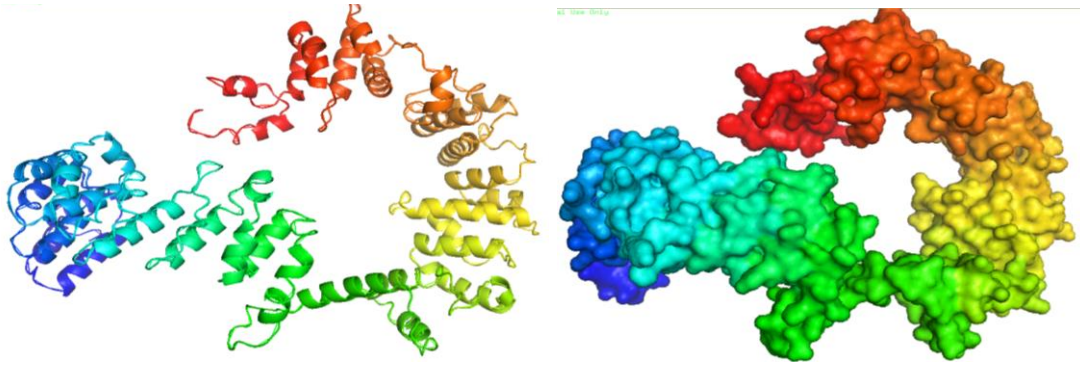


(a) (b)

Figure 1. (a) Ribbon shape of model and (b) Molecular surface of RNA-dependent RNA polymerase.

Central Asian republics), the Arabian Peninsula, Iraq, Pakistan, and Xinjiang Province in northwest China (Hoogstral, 1979; Burney et al., 1980). CCHF is a severe hemorrhagic fever in human with a high fatality rate more than 25% (Goodsell and Olson, 1990; Zanotto et al., 1996; Ergonul, 2006).

There is currently a re-emerging interest in reference to drug designing as being able to provide certain novel structures for the drug discovery effort and being particularly effective as antimicrobial leads (Newman et al., 2000; Dwivedi et al., 2011). One of the major worldwide facilitators of research and development of antimicrobial drugs and their application regimes is the global alliance specifically (Pauli et al., 2005; Dwivedi et al., 2011). It is certainly exciting to see natural products being the focus of such development efforts. To gain further insight into the relationship between the structure and biological activity, quantitative structure-activity relationships at the three-dimensional level (3D-QSAR), in general, is considered as a potential approach for developing newer drug leads based on small ligand structures (Cramer et al., 1988; Koonin et al., 1989; Talate et al., 1999). Further, RNA-dependent RNA polymerase is an essential protein encoded in the genomes of all RNA-containing viruses with no DNA stage. It plays a pivotal role in the biosynthesis of RNA strand harmonizing to a given RNA template. The RNA replication phenomenon is a two-step mechanism: (a) primarily, the initiation step of RNA synthesis initiates at or near the 3' end of the RNA template by means of a primer-independent (*de novo*), or a primer-dependent mechanism that utilizes a viral protein genome-linked primer; (b) Secondly, during an elongation phase, the nucleotidyl transfer reaction is recurred in view of generating the complementary RNA product (Sali et al., 1995; Kao et al., 2001). Therefore, in the present bioinformatics studies we are trying to build up a platform for exploring the specific ligand binding site on the active domain of RNA-dependent RNA polymerase for docking against a putative antiviral drug Ribavirin. These predicted binding sites will provide a sketch to design new and more effective antiviral drugs for this life threatening disease.

MATERIALS AND METHODS

The amino acid sequence of RNA dependent RNA Polymerase of CCHF viruses isolated earlier (Kao et al., 2001; Wishart et al., 2006) was retrieved from NCBI. The template for homology modeling was downloaded from protein data bank, and the protein was modeled using autodock 9v2.

Molecular modeling was performed in view of predicting their structures and establishing their function based on active site analysis based on already authenticated protocol (Castrignano et al., 2006; Wishart et al., 2006). The template selection was done for each of the targeted sequences, first aliening pair-wise to the sequences of already solved structures in Protein Data Bank (PDB) using BLASTp. Later on, the model building was carried out with the help MODELLER Version 9v2. The best template that came out of this investigation was 1U6G from *Escherichia coli*. Besides, docking was used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biochemical significance of molecular docking, considerable efforts had been directed towards improving the methods that pertains to predicting dependent RNA polymerase interaction with ribavirin using autodock 4.2.

RESULTS AND DISCUSSION

In the present study, RNA-dependents RNA polymerase sequence was retrieved from National Center for Biotechnology Information (NCBI) followed by modeling of its domain. PDB Blast results of the sequence showing alignment with various proteins were noticed to reflect that, the initial amino acids of the protein sequence was matched with high score but the query coverage was only 5 to 4%, due to the huge size of the sequence for the relevant improvement as far as its domain analysis was

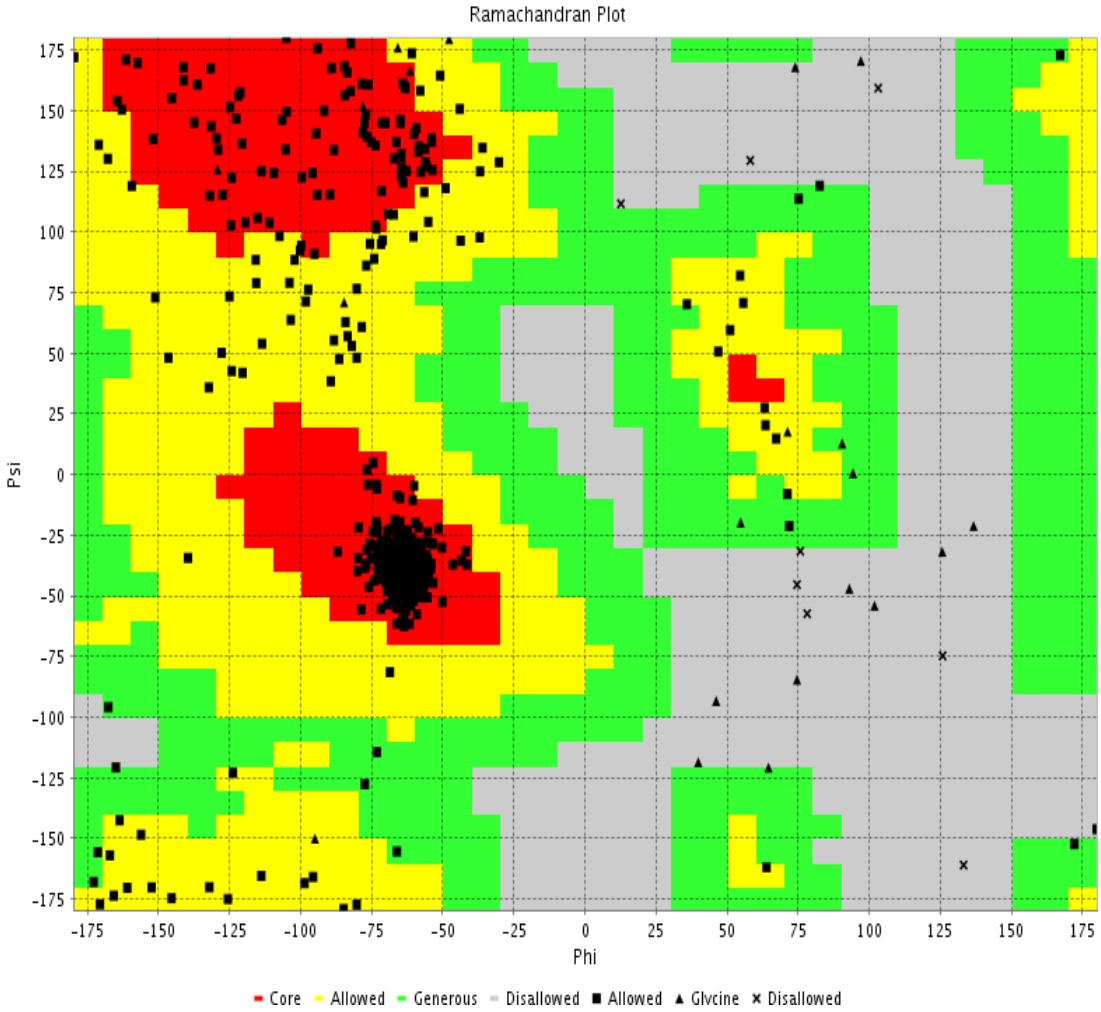


Figure 2. Ramachandran analysis of Model 3 showing about 97% amino acids lying in red and yellow meaning core and allowed region of the plot.

concerned.

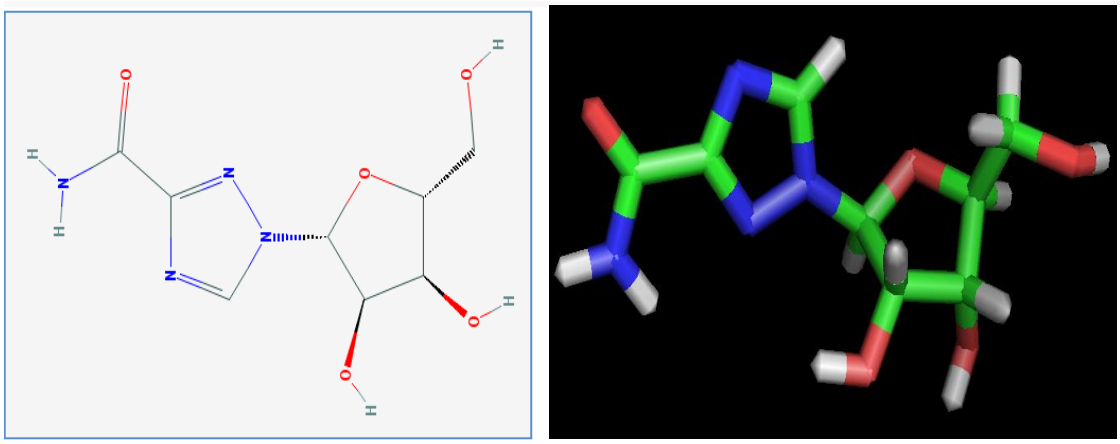
The domain OTU and Bunya_RdRp domains are available on the sequence. OTU family was shown to comprise a group of predicted cysteine proteases, homologous to the Ovarian tumor (OTU) gene in Drosophila. Members include proteins from eukaryotes, viruses and pathogenic bacterium. The conserved cysteine and histidine, and possibly the aspartate, were noticed to represent the catalytic residues in this putative group of proteases.

The 1U6G was taken into consideration as the template for homology modeling in Modeler 9v2 as shown in Figure 1 having Z score of -3.14 followed by Ramchandran plot (using the protocol published by Ramchandran et al., 1963; Figure 2) analysis of 97% reflecting the best possible model for further docking.

Ribavirin was designed using chemsketch software followed by the energy minimization (Figure 3). Docking the modeled protein with ligand showed the best active binding site were amino acid (MET197, VAL198, LEU200, VAL201, THR204, GLY205, ILE208, LEU209, GLN210, GLN211, LEU212, ALA213, PHE214, ALA215, LEU218, ALA226, PRO230, GLN233) and found to be conserved in forming the binding cavity for the ligand ribavirin. These findings can be well compared with the results obtained from the previous studies (Gilbert and Knight, 1986; Goldenberg et al., 2004; Jesper Brok et al., 2006).

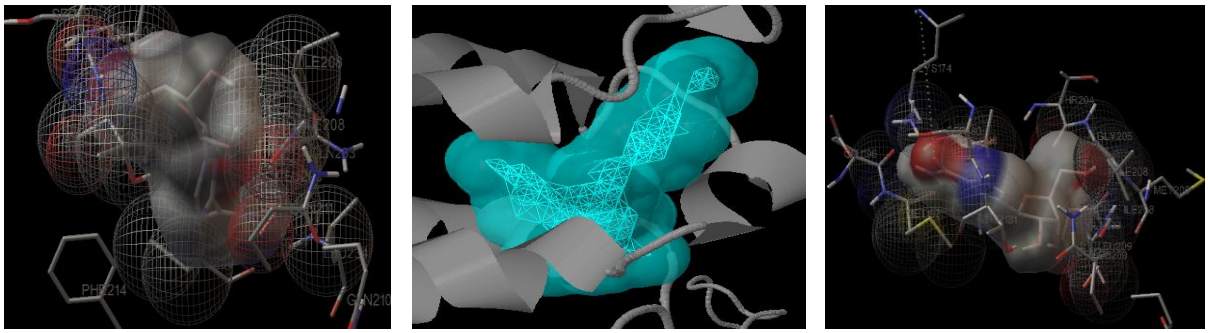
Further, drug bank data base contains nearly 4800 drugs entries including >1,350 FDA approved small molecule drug, 123 FDA approved biotech (protein/ peptide drugs, 71 nutraceuticals and more than 3243 experimental drugs). Additionally more than 2500 non redundant protein (that is, drug target) sequence are linked to these FDA approved drug entries for the docking analysis of Ribavirin which has been searched using drug data bank. These are the residue which contributes to the active site of the protein known to act as a target for drug binding.

The active site on the molecule was assessed by the Q



(a) (b)

Figure 3. (a) 2D Structure of the Ribavirin and (b)3D Structure of the Ribavirin.



(a) (b) (c)

Figure 4. (a) The active site on the modeled protein which consist of above mentioned residues in the pocket. (b) Docked model of RNA-dependent RNA polymerase and Ribavirin. (c) Ribavirin interacting with residues in minimum energy conformation.

site finder server reflecting the site volume about 536 Å, and the total protein volume: 56916 Å (Figure 4) probably may be used to probe the finding of active site by probe rolling mechanism. Further, obtaining the estimated free energy of binding being 392.36 kcal/mol reveals the stability of docked molecule. Taken together the data obtained from the overall present study and the studies carried out by the previous workers (Gilbert and Knight, 1986; Goldenberg et al., 2004; Laurie and Jackson, 2005; Jesper Brok et al. 2006) along the same notions and objectives would certainly be helpful in broad virtual screening of inhibiter of the protein and can be further implemented in future drug designing.

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