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RESEARCH ARTICLE

HOMOLOGY MODELING AND BINDING SITE PREDICTION OF VP30 PROTEIN INVOLVED IN EBOLA VIRUS

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ABSTRACT

The Ebola virus (EBOV) genome encodes for several proteins that are necessary and sufficient for replication and transcription of the viral RNAs; NP, VP30, VP35, and L. VP30.VP30 binds to the RNA at the first gene start signal to initiate transcription. In the present study we used different *In Silico* tools and technique to analyze protein sequence of VP30 protein retrieved from major protein resources, prediction of three dimensional structures and its binding site prediction. However, we predicted the three dimensional structure of VP30 protein by Swiss model server and Ramchandran Plot analysis. Our work suggests that VP30 protein can acts as target for the inhibition of Ebola virus. The further study of VP30 protein used in the molecular docking and structure based drug designing to inhibit in Ebola virus.

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INTRODUCTION

Ebola viruses (EBOV) are no segmented, negative-stranded RNA viruses, which together with Marburg virus constitute the family Filoviridae. Filoviruses cause severe and lethal hemorrhagic fevers in humans and nonhuman primates and, as such, are classified as biosafety level 4 (BSL-4) agents (Sinu P. John). After entry into the host cell, the EBOV envelope fuses with host cell membranes to release the nucleocapsid into the cytoplasm where transcription and replication take place. Initial transcription of the newly entered encapsidated RNA genome is entirely accomplished by the nucleocapsid proteins that are associated with the intruding virus (primary transcription). Transcription is regulated by conserved transcription start and stop signals at the viral gene borders (Nadine Biedenkopf *et al*). For transcription of the viral genome four viral proteins are essential: the nucleoprotein NP, the polymerase L, the polymerase cofactor VP35, and VP30. VP30 represents an essential Ebola virus-specific transcription factor whose activity is regulated via its phosphorylation state.Currently, neither an approved vaccine nor antiviral therapy is available for humans.

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The enveloped EBOV particle is composed of seven structural proteins, five of which form the helical nucleocapsid that represents the template for viral transcription and replication. The viral genome is encapsidated by the major nucleocapsidprotein NP, and VP35, VP30, and VP24 interact with NP to form the mature nucleocapsid Transcription is regulated by conserved transcription start and stop signals at the viral gene borders. The gene start signals are part of RNA secondary structures, and it has been proposed that VP30 binds to the RNA at the first gene start signal to initiate transcription. In addition, VP30 was shown to be important for transcription reinitiation of subsequent genes (Bettina Hartlieb). Phosphorylation of VP30 positively regulates its binding to NP and negatively regulates its transcriptional activity. In addition, enhancement of transcription by VP30 requires a putative RNA secondary structure located within nucleotides (nt) 54 to 80 of the leader region.

Deletion of the predicted RNA secondary structure permits VP30-independent transcription of viral messengers. These reported activities of VP30 suggest the possibility of a direct interaction of VP30 with EBOV RNA in its role in transcription. Recent publications with the minigenome system for EBOV suggest at least two possible mechanisms that VP30 may use in its transcriptional regulatory role. One possible mechanism could be that VP30 interacts with one or more of the other nucleocapsid proteins, polymerase, NP or VP35, and

promotes increased stability of the transcriptional complex, VP30 may interact directly with viral RNA(s) to regulate transcription (Sinu P. John). In the present study we analyze protein sequence and predicted the 3 dimensional structure of VP30 protein using various bioinformatics tools. And we have also predicted the active/binding sites for the protein. These sites can be further use for the drug designing purpose for VP30 protein.

MATERIALS AND METHODS

Protein sequence retrieval and primary analysis

Protein sequence of protein minor nucleoprotein vp30 was retrieved from Uniprot kb database. The physicochemical analysis were calculated by ProtParam tool (http://web.expasy.org/protparam/), including *pI*, total number of negatively and positively charged residues, the instability index (II), aliphatic index, and grand average of hydrophilic (GRAVY).

Structural characterization

Secondary structure prediction was performed by using SOPMA (Geourjon and Deléage, 1995) server (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html). SOPMA is using homologue method of Levin *etal*.According to this method, short homologous sequence of amino acids will tend to form similar secondary structure.

Homology modeling and model evaluation

Homology modeling was used for determining 3D structure of protein. Then, BLASTP was performed against PDB (Protein Databank, Bernstein *et al*., 1977) to retrieve the best suitable templates for homology modeling. Preferred hit contains maximum identity and lowest e-value that it was used as a template. The modeling of the 3D structure of the protein was performed by using Swiss-Modeler (http://swiss-model.expasy.org/) program (Arnold *et al*., 2006; Bordoli *et al*., 2009). After modeling, the quality and validation of the model was evaluated by Ramachandran plot analysis using PDBsum server (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/).

Binding site prediction

The binding site of VP30 protein was predicted by PDBsum server (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/). The binding site shows the small pockets of the tertiary structure where ligands bind to using the weak forces.

RESULTS AND DISCUSSION

Protein sequence retrieval and primary analysis

The physicochemical analysis of vp30 protein was performed using Protparam and results were shown in Table 1. Vp30 protein contains 281 amino acids with molecular weight 31649.8 dalton.

Table 1. Physico-chemical properties of vp30 protein

Parameters	Values
Molecular weight	31649.8
Theoretical pi	9.40
Instability index	57.37
Extinction coefficients	21805
Total number of negatively charged residues (Asp + Glu):	25
Total number of positively charged residues (Arg + Lys):	35
Aliphatic index:	69.11
GRAVY	-0.777

Table 2. Secondary structure of vp30 protein using SOPMA

Parameters	Values
Alpha helix	30.25%
Beta sheet	7.83%
Coils	52.31%

Protparam tool computed that the Vp30 protein is basic in nature and unstable on the basis of parameters Theoretical pi and instability index. According to the GRAVY index protein is hydrophilic. The aliphatic index of a protein is 69.11which defined as the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). The total number of positively charged residues (Arg+Lys 35) was found higher than the total number of negatively charged residues (Asp+Glu 25).

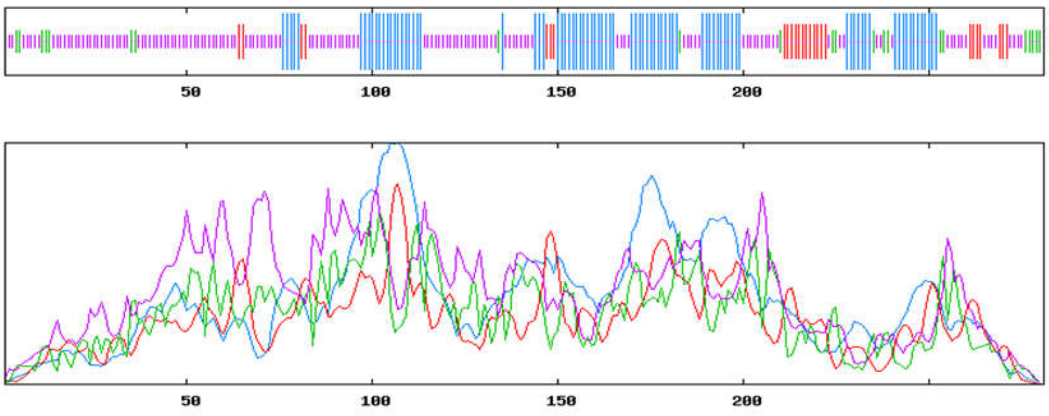

Figure 1. Secondary structure of vp30 protein using SOPMA

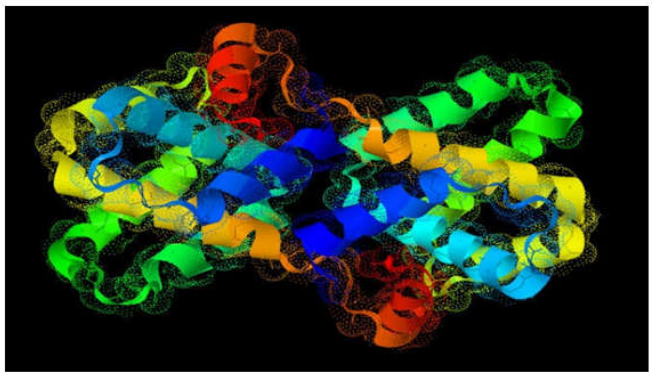

Figure 2. Predicted 3D structure of protein vp30 using SWISS-Model

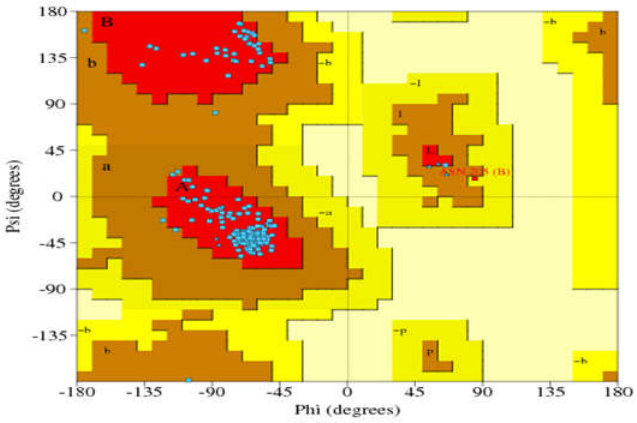

Figure 3. Ramachandran plot analysis

PROCHECK statistics

1. Ramachandran Plot statistics

		No. of residues	%-tage
Most favoured regions	[A,B,L]	219	96.9%
Additional allowed regions	[a,b,l,p]	6	2.7%
Generously allowed regions	[~a,~b,~l,~p]	1	0.4%
Disallowed regions	[XX]	0	0.0%
Non-glycine and non-proline residues		226	100.0%
End-residues (excl. Gly and Pro)		4	
Glycine residues		16	
Proline residues		4	
Total number of residues		250	

Figure 4. Procheck analysis

Structural characterization

The secondary structure of the protein was predicted using SOPMA server (Table 2 and Figure 1). It was observed that random coil was predominant (52.31%), followed by alpha helix (30.25%) and extended strand (7.83%). Random coils have important functions in proteins for flexibility and conformational changes such as enzymatic turnover (Buxbaum, 2007).

Homology modeling and model evaluation

The SWISS-MODEL homology modeling program was used for the predicting of three dimensional structure of the vp30 protein (Figure 2). PDB id 3V7O (Crystal structure of the C-terminus domain of Ebola virus) was selected as template with 37.50% sequence identity to query sequence. The quality and validation of the model was evaluated by Ramachandran plot analysis using PDBsumserver (Figure 3 & 4). Ramachandran plot analysis showed that only 1.4% residues in outlier region, 3.2% allowed region and 95.4% in favored region, indicating that the models were of reliable and good quality.

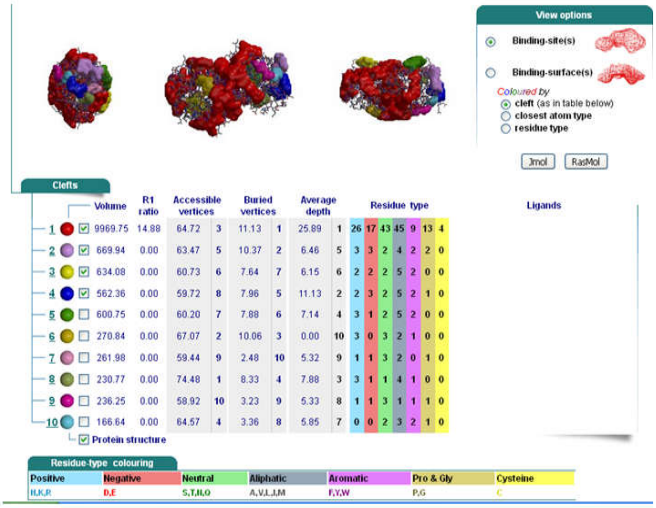

Figure 5. Binding site analysis of predicted structure of VP30 protein

Binding site prediction

The predicted structure of VP30 protein was further studied for its binding site prediction at PDBsum server. This server predicted that predicted structure contains three binding sites (Figure 5).

Conclusion

The VP30 protein is involve in the transcription of Ebola virus. The present study we analyzed the physicochemical properties of VP30 protein by using Protparam tool. The 3D structure of protein was predicted using SWISS MODEL server. The final model was further evaluated by using Procheck and Ramachandran plot analysis. Binding site of the protein was studied using PDBsum database. From the present study it has been concluded that VP30 protein can be used as target for the inhibition of Ebola virus. The molecular structural insight encompasses to the development of new drug for inhibition VP30 protein.

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