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An *In Silico* Approach: Homology Modelling and Docking Studies of Rabies Virus Glycoprotein with Salviifoside A of *Alangium salviifolium*

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Abstract: ***The genome of rabies virus encodes five proteins; the nucleoprotein, the phosphoprotein, the matrix protein, the glycoprotein, and the RNA-dependent RNA polymerase. Among these, the glycoprotein is the most important as it is the major contributor to pathogenicity and virus neutralizing antibody response. Keeping in mind that glycoprotein is the only protein exposed on the surface of virus and is thought to be responsible for the interaction with the cell membrane, it was attempted to target glycoprotein by a ligand salviifoside A of Alangium salviifolium which blocks its active site, so that it may be possible to prevent the spread of virus into the host. The ligand salviifoside A of Alangium salviifolium was retrieved from Pubchem and docked with modelled Rabies Virus Glycoprotein (RVG). This knowledge may be important for the further research studies and aid in treatment of rabies and other viral diseases in the future.***

Keywords: Rabies Virus Glycoprotein (RVG), Encode, Pathogenicity, Modelled, Docking

1. Introduction

Rabies, severe encephalitis of mammals, is caused by the members of the lyssavirus genus of the Rhabdoviridae family, order Mononegavirales. The disease caused by rabies virus (RV) is fatal once clinical symptoms appear in the form of encephalomyelitis in several species of mammals including humans [1]. Human rabies is mainly transmitted through a rabid dog bite in the developing world, of which 94-98% of deaths are due to canine rabies [2]. World-wide human mortality from rabies is estimated to be 55000 deaths per year with 56% of these deaths estimated to occur in Asia and 44% in Africa. In India alone, 18500 people die and some 70,0000 people take rabies prophylaxis each year following exposure to rabid animals.

The genome of virus is about 12 kb that encodes five proteins; the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the glycoprotein (G), and the RNA-dependent RNA polymerase (L). The viral RNA, which is always encapsidated by N, forms the ribonucleoprotein (RNP), which is the template for viral replication and transcription [3]. The RVG, which is organized as a trimer, is the major contributor to pathogenicity. It interacts with cellular receptors [4], mediates pH-dependent fusion, and promotes viral entry from a peripheral site into the nervous system [5]. Moreover, RVG is involved in the trans-synaptic spread within the central nervous system [6,7]. Although RV pathogenicity is a multigenic trait, the G is major contributor to the pathogenicity of a particular Rabies Virus [8]. The efficient interaction of RVG with putative host cell receptors can promote effective virus uptake resulting in increased virulence. There have been recent important advances in our understanding of how rabies virus spreads and causes disease in its hosts. Because current approaches to the management of human rabies have proven unsatisfactory, more research is needed in good experimental animal models in order for us to better understand the pathogenesis of this ancient disease.

Tribal of Vindhya and Satpura region use this plant in treatment of various problems such as insect and scorpion sting, dog bite, arthritis and fever [9].The plant *Alangium salviifolium* has been reported to contain various biologically active phytochemicals such as alangine, ankorine, tubulosine, alangicine, salsoline etc. Recently three phenolic glycosides, salviifoside A, salviifoside B, salviifoside C, along with three known compounds salicin, kaempferol, and kaempferol 3-O-β-Dglucopyranoside were isolated from the leaves of *Alangium salviifolium*. Salviifoside were also revealed to possess anti-inflammatory activity [10].The present study was attempted to model the RVG from organism Rabies virus (strain India) and dock it with the phenolic glycosides salviifoside A of *Alangium salviifolium* the pathogenic glycoprotein with identification of a suitable ligand in order to search a cure for this disease by development of novel therapeutics based on the information of docked molecule with virus glycoprotein. If the glycoprotein can be targeted by a ligand which blocks its active site, it is possible to prevent the spread of virus into the host. This knowledge may be important for the development of novel therapies for the treatment of rabies and other viral diseases in the future.

2. Materials and Methods

In this investigation an attempt was made to model the RVG followed by validation of the obtained model and docking it with the phenolic glycosides compound salviifoside A of *Alangium salviifolium*.

2.1 Preparation of ligand molecule

The NCBI Pubchem chemical compound was used to select the chemical compound. The structure of ligand molecule was retrieved from Pubchem as 3-D SDF format and converted into MOL2 format and PDB format using Open Babel software [11]. Pubchem Id of Salviifoside A: CID 45273489(https://pubchem.ncbi.nlm.nih.gov/#).

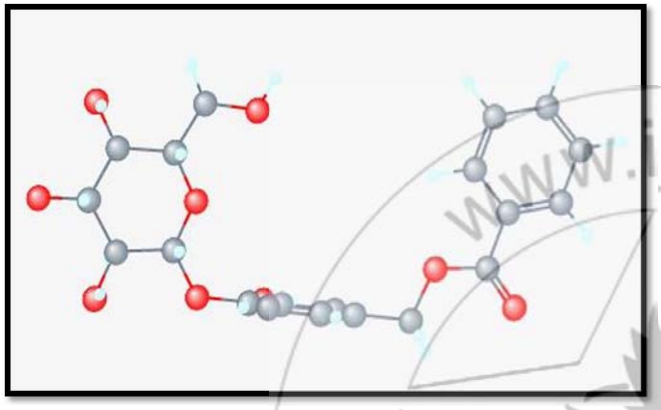


Figure 1: 3-D Conformer of Salviifoside A (Pubchem ID-CID 45273489)

2.2 Preparation of receptor molecule using homology modelling

For the preparation of receptor molecule, the target protein sequence RVG was retrieved from Uniprot(Accession code A3RM22) [12]. The UniProt accession code of the target protein was given as input data in Swiss-Model online server in automated mode [13]. The pipeline will automatically select suitable templates based on the Blast, E-value limit, experimental quality, bound substrate molecules, or different conformational states of the template. Finally as a result five models were generated using Swiss-model.

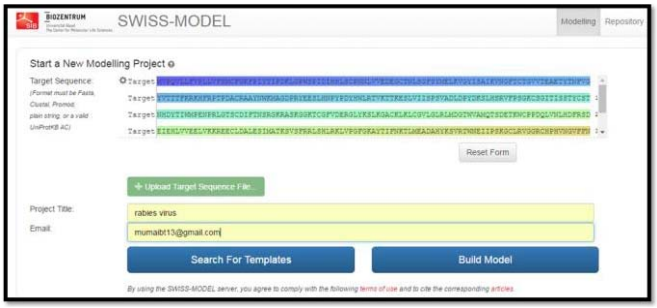


Figure 2: Swiss-Model (automated mode) - RVG as input sequence

2.3 Validation of predicted structure

Using PROCHECK interactive server the stereochemical quality of the protein structure was analyzed residue-by-residue geometry and compared with stereochemical parameters derived from well-refined, high-resolution structures [14]. Finally the 3D models were validated on the basis of Ramachandran plot statistics. From the generated models, the one with highest number of residues in the allowed region and minimum number of residues in the disallowed region were considered as the suitable model. The best model was then considered for further analysis.

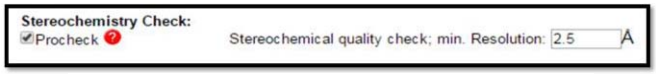


Figure 3: Stereochemical analysis with Procheck

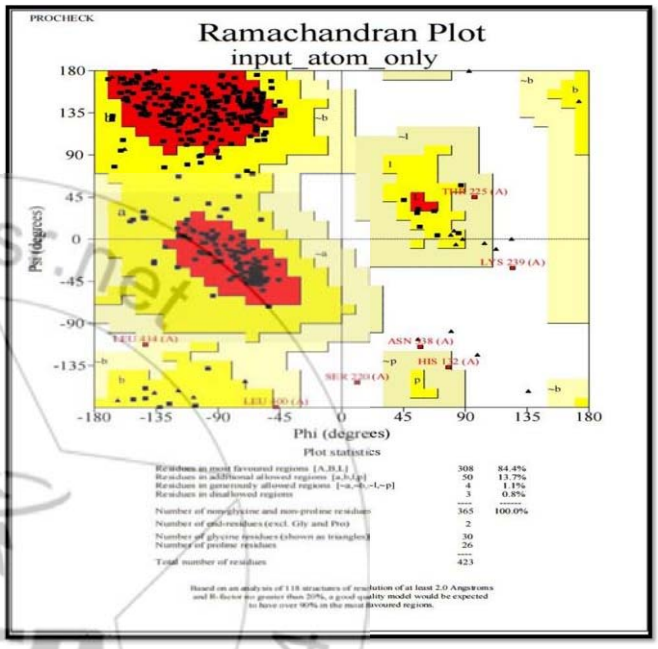


Figure 4: Validation of model using Ramachandran plot

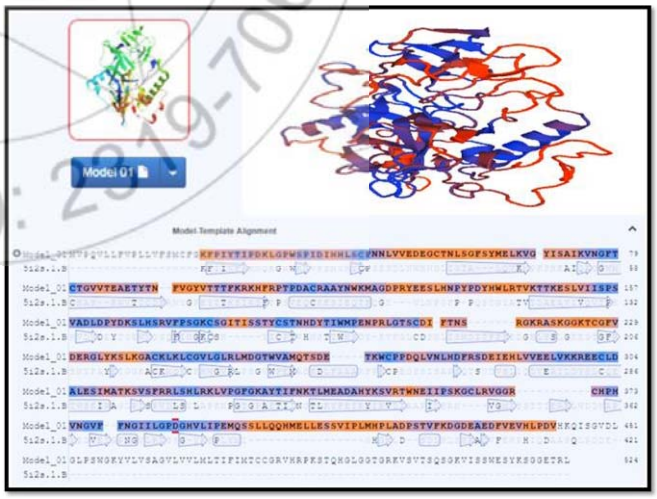


Figure 5: Model 1 validated from Procheck result

3. Docking

Docking was carried out for the retrieved ligand molecule and the modelled protein receptor in PDB format, FASTA sequence for PatchDock and SwissDock respectively. PatchDock, a web service to predict the molecular interactions that may occur between a target protein and a

small molecule based on shape complementarity principles (http://bioinfo3d.cs.tau.ac.il/PatchDock/).

SwissDock, a web, dedicated to the docking of small molecules on target proteins is based on the EADock DSS engine, combined with setup scripts for curating common problems and for preparing both the target protein and the ligand input files (http://www.swissdock.ch/docking). Molecular docking studies help in prediction of the preferred orientation of a ligand with the binding site on a protein. Molecular docking was used to determine appropriate binding orientations and conformations of various chemical compounds at the target site. After docking, all the legend confirmations were ranked on the basis of their binding energy. Protein and ligand interactions were determined using Discovery Studio 4.0 (http://www.accelrys.com), which explained the active binding sites in receptor protein and show best docking confirmation.

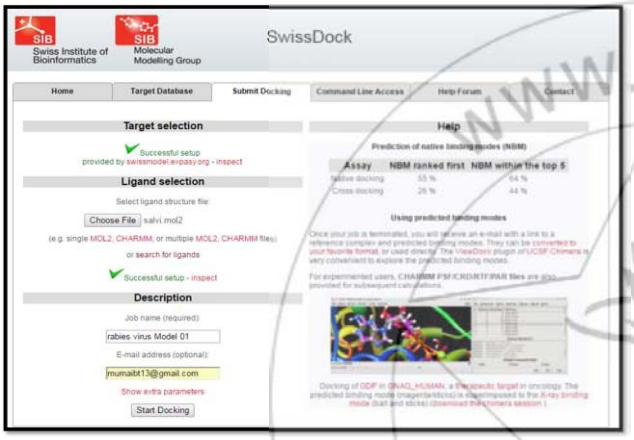


Figure 6: Input files – Target sequence and ligand molecule uploaded in SwissDock

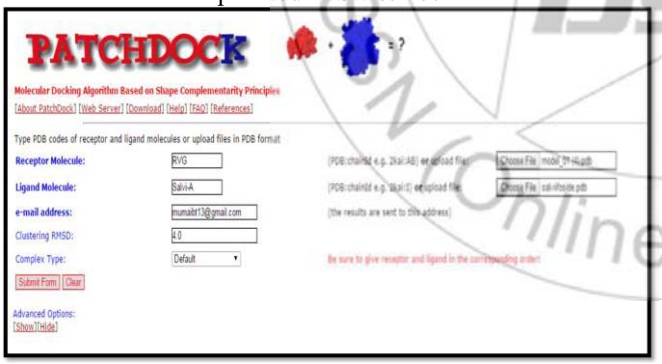
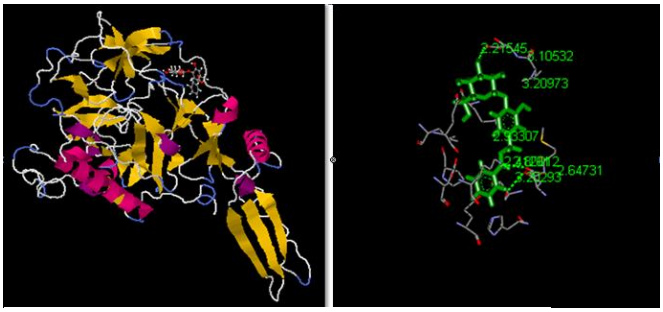


Figure 7: Input files – Target sequence and ligand molecule uploaded in PatchDock



(a) SwissDock output visualized in Pymol (b)PatchDock output visualized in DS visualizer

Figure 8: Interaction of RVG with salviifoside A molecule

Table 1: Docking Energies for selected ligand molecules with different docking tools

Ligand Compound Name and Pubchem ID	Receptor Protein Name	Swiss Dock Kcal/mol	Patch Dock Kcal/mol
Salviifoside A CID 45273489	Rabies Virus Glycoprotein RVG	-7.83	-47.78

4. Result

From the protein modelling studies of the present research investigation we retrieved the amino acid sequence of RVG (Strain India) from Uniprot. The total number of amino acids of RVG protein is 524 residues and mass 58,365 Da. By using our sequence of RVG from Indian strain (Accession number A3RM22) of rabies virus as a query sequence, many possible structures were generated by homology modelling.

In protein modelling studies, we used an automated protein modelling server named SwissModeller in which the query sequence is first attempted modelled using the profile scoring function suitable for close homology modelling.

Among these the best structure (having minimum energy) was selected as a target receptor for ligand salviifoside A. Validation of modelled structure was obtained from Procheck and the stereochemical quality of the modelled protein was checked by Ramchandran plot which showed 84.4% residues in most favoured regions and 13.7% residues in additionally allowed region.

PatchDock and SwissDock online docking Servers were used in the molecular docking on geometry-based algorithm. This server was applied to recognize the binding scores and binding residues. The docking results were obtained through the email, whereas RVG showing different binding score against salviifoside A. The binding affinity of RVG is presented in the docking score which is 5116, for salviifoside A in SwissDock.

5. Conclusion

The development of novel compounds with biological activity is an urgent need. In the present study phytocompounds salviifoside A of *A. salviifolium* were successfully docked into the RVG protein for interaction study and development especially new compounds which are more important for the discovery of new hits using molecular methods. Though the binding pattern of ligands with RVG differed respect to Atomic contact energy (ACE), fullfitness score and estimated delta G values substantiate the hypothesis that salviifoside A has the potentiality to selectively inhibit the RVG. Hence, it is concluded that salviifoside A could be a potent anti-inflammatory target molecule against RVG which aid in the performance of further research.

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