Article

Theoretical modeling of Replication Association Protein of Indian Cassava Mosaic Virus and its docking with N-Acetyl D-Glucosamine

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ABSTRACT

The Bean golden mosaic virus (BGMV), also known as the Begomovirus, belongs to the family Geminiviridae and is transmitted through the whitefly. One of the forms of it is Indian cassava mosaic virus (ICMV) which adversely affects the yields of tomatoes, guavas, green chillies, potatoes, etc. ICMV has been modelled theoretically in the present work with the help of tools of homology modelling. The latter is concerned with insilico approaches to build, predict and analyse the 3-D structure of proteins (for which the crystal structure is unavailable). The closest homologue of Association Protein of Tomato was 1L2M_A, with the highest sequence identity. Both consensus and chimera models were made and studied. The best one was then used for the docking studies. Thereafter, the putative binding sites to be attacked by various inhibitors were located and then docked with N-Acetyl-D-glucosamine (NAG). NAG was optimized using B3LYP/6-31G(d,p) methods and its properties are studies. The docking scores of NAG on the active sites of Replication Association Protein of Indian Cassava Mosaic Virus were checked and it was found that NAG proves to be an effective inhibitor for ICMV.

Keywords: Cassava, Begomovirus, Homology

INTRODUCTION

Begomoviruses have come out as a serious threat to a variety of economically important plants. Begomoviruses belong to the Geminiviridae family and is transmitted through white flies (*BemisiatabaciGennadius)*. It generally infects dicots in tropical and temperate climates.[2-4] They belong to either the new world viruses or the old world viruses. The former have a bipartite genome (DNA-A and DNA-B) whereas the latter has either a monopartite (DNA-A) or a bipartite genome.[5-7] Presently, Begomoviruses are associated with sever diseases in a wide range of crops such as cassava, cotton, legumes, grains and vegetables[1]. They have also been found to infect tomatoes, okra, chillies, beans, cucurbits and weeds. Begomoviruses are believed to have a recombination tendency and development of new viruses is found due to acquisition of added DNA components.[8] Therefore, it has become very important in day to day life to manage viruses to control and minimize the diseases caused by them. It has been stated that virus infected plants cannot be controlled or cured by any chemical treatment in the field.[9] There are many conventional and non-conventional methods to control the viral diseases over years. But the best possibility to explore a cure for Begomoviruses is yet to be established.[10]

Many strategies have been developed for integrated disease management by spray of oils, pesitcides, viricides and botanicals which reduces the yield loss for various viral diseases.[11-18] Management of begomoviruses through chemical means is done by controlling the population of transmission vectors.[10] Through this work, we intend to prove that chemicals or small molecules are capable of inhibiting the action of begomoviruses also. We begin our work by picking up an economically important plant i.e. Cassava. Cassava is a species not native to the Old World and which has been introduced from the New World in the 16th century. There is no associated cassava infecting begomovirus in the New World, indicating that the cassava mosaic disease (CMD) causing viruses are local viruses adapted to infect the cassava plant. This can be supported by the similarity of the ICMV to the other Begomoviruses in the subcontinent, affecting other crops, over the other CMD causing virus in Africa, the African Cassava Mosaic Virus (ACMV). Furthermore the Old World Begomoviruses all have the AV2 gene absent in the New World Begomoviruses, indicating a convergent evolutionary pathway.[19] The cassava crop, cultivated in the southern and central India, is affected mainly by the Indian Cassava Mosaic Virus (ICMV) and the Sri Lanka Cassava Mosaic Virus (SLCMV), identified as being from Sri Lanka.[19,23] The origin of the SLCMV has not been determined- if the virus

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originated in Sri Lanka and was transported to India or vice versa.[22] Both the viruses are distinct from the other Begomovirus affecting cassava in Africa, the African Cassava Mosaic Virus (ACMV).

The virus samples isolated indicate that the ICMV distribution is restricted to the northern and central areas of Kerala and Tamil Nadu while SLCMV was found in all the CMD affected areas (Patil et al). The CMD viruses isolated from India in the period 2001-02 showed similarities to the ICMV and the SLCMV, majority showing similarities to the SLCMV. The variants isolated from the Indian subcontinent showed evidence of DNA recombinations between ancestors of ICMV and SLCMV.[22] Recombination is the process where, during replication, nucleotide sequences from one strand is incorporated into the genome of another individual strand. Recombinations caused in CMD viruses would become prevalent in the infecting populations only if the recombination resulted in an advantage.[24-29]

Diversity in the virus resulted from mutations in the genome of the virus-point mutations and due to the tendency of the virus to undergo recombinations and exchange their DNA components.[30-31] Begomoviruses possess two DNA particles- DNA A encoding functions associated with viral replication and encapsidation while the DNA B fragment encodes for movement functions. It has been theorised that the SLCMV was originally a monopartite virus which has evolved into a bipartite virus by capturing its DNA B component during a recombination event with the ICMV's DNA B. Evidences provided for this are- the DNA A component of SLCMV shows the propensity to cause to cause aggressive infection resembling those caused by a monopartite virus (upward leaf-curl, vein thickening) in the absence of the DNA B component, as seen by mechanical agroinoculution in *N.bethiamiana*, and the high resemblance of the SLCMV's DNA B to that of the ICMV.[30-33] The DNA A components of the Begomoviruses usually remain more highly conserved than the DNA B components but in case of SLCMV and ICMV, the DNA B components resemble each other greatly outside of their common region, but the similarities reduce in the common region, especially those above the stem loop. However this region above the stem loop is highly conserved between the SLCMV components. This suggests the recombination of the SLCMV DNA A component with the DNA B component of the ICMV, giving rise to the SLCMV DNA B component. The recombination is thought to have been a recent event due to the presence of the DNA B sequences external to the common region remaining conserved. Thus the progenitor of the SLCMV possibly still exists in association with a satellite DNA, and the SLCMV may represent an intermediate in the evolution of a monopartitegeminivirus to a bipartite one.[25-30] However, during infection of cassava plant, the DNA A of SLCMV is always associated with the DNA B component.

Such recombinations are facilitated in case of mixed infections in a plant. Viable pseudorecombinations can also be seen in case of infection by less closely related SLCMV and ACMV in *N.bethiamiana*, where the DNA A of SLMCV and DNA B of ACMV generate a mild infection with symptoms resembling infection caused by either of the viruses. The mild phenotype indicates lesser degree of

compatibility between the genomic components of the two viruses.[25-29] In India, studies have shown presence of both ICMV and SLCMV in cassava crops. SLCMV has shown tendency to acquire DNA components from other viruses and evolve. Latest studies have shown that both ICMV and SLCMV are present in cassava crops in India. SLCMV has the capability to acquire DNA components from other viruses and then subsequently evolve. Therefore cases of mixed infections can potentially cause generation of new virus species, resulting in an epidemic similar to the African CMD epidemic caused by a highly virulent strain of CMD virus. This can now be determined using chimera of two distinct begomoviruses.

Computational details

In the present work we used different bioinformatics tools and biological databases for the replication association protein of Indian Cassava Mosaic virus which is a form of Begomovirus, like GenBank-NCBI, PDB (Protein Data Bank), uniprotKB results, etc. Query Q82676 was picked up from uniprotKBresults which represents Indian Cassava Mosaic Virus with genes AL1 and AC1. The length of the query picked up is 351 residues. Homology modelling was performed for this query which basically consists of four sequential steps: template selection, target template alignment, model construction and model assessment[33]. Models were built and refined using Prime**.** Prime of Schrödinger Release 2015-4 was used for building all the models based on single and multiple templates (consensus and chimera models).[35-37] Glide (Schrödinger, Inc.) calculations were performed to estimate the ligand binding energie.[38-40] The ligand molecule i.e. N-Acetyl D-Glucosamine was subjected to energy minimization using B-3LYP/6-311g(d,p).[42] After ensuring that the protein and ligands were in the correct form for docking, the receptor grid files were generated using a grid-receptor generation program. To soften the potential for non-polar parts of the receptor, the van der Waal radii of receptor atoms were scaled by 1.00 with a partial atomic charge of 0.25. A grid box of size $56 \times 56 \times 56$ Å with coordinates X = 38.299, Y = 35.126 and Z = 90.3588 Å was generated at the centroid of the ligand. The ligands were docked into the active site using the 'extra precision' XP Glide algorithm. Based on the model energy score (Emodel), which combines the van der Waals and Coulomb energies, lipophilic contact and hydrogen bond terms, penalties for buried polar groups and freezing rotatable bonds, as well as the polar interactions at the active site, with the nonbonded interaction energy and the excess internal energy of the generated ligand conformation, a single best pose was generated for each ligand. For the calculation of the free energy of binding (FEB) of the ligand i.e. N-Acetyl D-glucosamine with Homology models of ICMV, only the best scoring pose for the ligand was taken into consideration. The basic idea of docking was picked up and glide scores were checked.[41]

RESULT AND DISCUSSION

Homology Modeling
The protein template sequence of Indian Cassava Mosaic virus was searched from uniprot Knowledge based search and mined into the FASTA format as follows:

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>sp|Q82676|REP_ICMV Replication-
associated protein OS=Indian cassava
mosaic virus GN=AC1 PE=1 SV=1
MSPPKRFQINAKNYFLTYPRCSLTKEEALSQIRNFQTPTN
PKFIKICRELHENGEPHLHV
LIQFEGKYKCQNQRFFDLVSPTRSAHFHPNIQGAKSSSDV
KSYIDKDGDTWRWGTFQIDG
RSARGGQQSANDAYAAALNSGSKSEALKILRELAPRDYLR
DFHHISSNLDRIFTKPPPPY
ENPFPLSSFDRVPEELDEWFHENVMGRARPLRPKSIVIEG
DSRTGKTMWSRALGPHNYLC
GHLDLSPKVYNNDAWYNVIDDVDPHYLKHFKRIHGGPEDW
QSNTKYGKPVQIKGGIPTIF
LCNPGPNSSYKEFLDEEKNSALKAWALKNATFISLEGPLY
SGTNQGPTQSC
```

The FASTA sequence was uploaded in the Multiple sequence Viewer and then a Blast Search was performed using remote NCBI server from all the NCBI PDB files. The search gave results as shown in Table 1.

Table 1. Calculated identity, similarity and homology parameters for blast search done on the Query Q82676

S.No.	PDB-ID	identity	similarity	homology
1	1L2M_A	4.23729	12.7119	14.4068
2	4BKS_C	0.83333	5.83333	5.83333
3	4AWJ_C	0.83333	5.83333	5.83333
4	4WQO_A	0.83333	5.83333	5.83333
5	4B9K_C	0.83333	5.83333	5.83333
6	1LQB_C	0.83333	5.83333	5.83333
7	3ZRC_C	0.83333	5.83333	5.83333
8	1VCB_C	0.83333	5.83333	5.83333
9	1D4B_A	1.66667	5.83333	6.66667
10	2A7W_A	2.5	5	9.16667
11	3E4Z_A	3.33333	11.6667	11.6667
12	2E4Z_A	5	13.3333	14.1667
13	1C1K_A	4.5977	14.9425	11.4943
14	3OIC_A	4.5977	12.6437	18.3908

HOMOLOGY MODELS

Single Template (using 1L2M)

Based on the Blast search results, IL2M (Tomato yellow leaf curl virus - Sardinia) is found to possess maximum sequence identity. Tomato yellow leaf curl virus - Sardinia possesses 118 residues and is a replication protein. It is a Minimized Average Structure of the N-terminal, DNA-binding domain of the replication initiation protein from a geminivirus. The uniprot id is P27260. Its respective sequence was incorporated and a single template homology model was constructed. The obtained model is shown in Figure 1.

The single template option was used to build a model for a single chain from a single template. The template was selected from the available sequences in the MSV, and then the PDB structure imported.

Figure 1. ICMV model based on Tomato yellow leaf curl virus.

In any polypeptide chain, the main chain N-C α and Cα-C bonds relatively are free to rotate. These rotations are represented by the torsion angles ϕ and ψ, respectively.

Computer models of small polypeptides were used initially by Ramachandran to systematically vary ϕ and ψ with the objective of finding stable conformations. For each conformation, the structure was examined for close contacts between atoms. Atoms were treated as hard spheres with dimensions corresponding to their van der Waals radii. Therefore, ϕ and ψ angles which cause spheres to collide correspond to sterically disallowed conformations of the polypeptide backbone.[43-46] The Ramachandran plot obtained for the single template model is as shown below in Figure 2.

The respective Ramachandran plot shows a good concordance showing that the model can be utilized for getting an idea of how a ligand would dock the ICMV. The putative binding sites were mapped and were docked with N-Acetyl Glucosamine after the optimization of latter. The best site for docking was chosen by checking the highest druggability score. The site is shown in Figure 3 and Figure 4. The glide score turned out to be -5.729 which proves NAG to be a good inhibitor for this model. We then move on to check its inhibition with other homology models created.
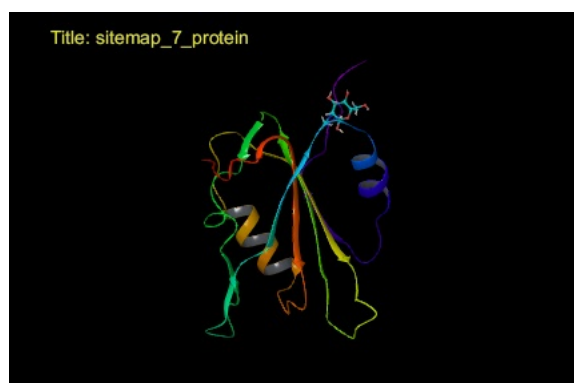
Multiple Template (Chimera Model)

From Table 1, two suitable template models were suitably picked up to build a chimera model. The template models chosen for study in the present case are 1L2M (Tomato yellow leaf curl virus – Sardinia) and 3OIC (Crystal Structure of Enoyl-ACP Reductases III (FabL) from B. subtilis (apo form)) which showed maximum homology. The homology model obtained for ICMV is given in figure 5 and the respective Ramachandran plot is shown in figure 6.

The Ramachandran plot obtained in this case lies just within the acceptable limits of >90% accuracy. The number of residues in the disallowed (white) region have increased

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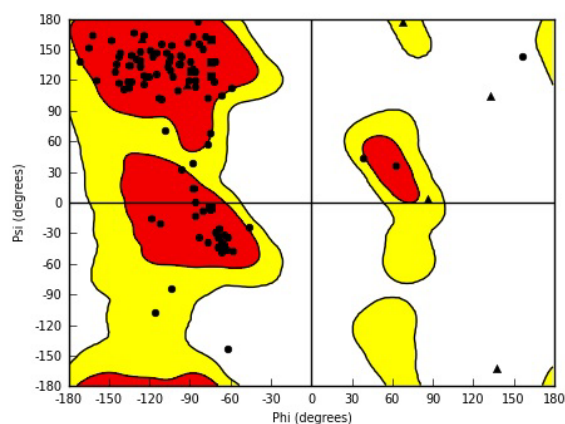
Figure 2. Ramachandran plot showing the accuracy of the ICMV model made to be >95% accuracy.

**Figure 3.** Single template ICMV model docked with NAG.
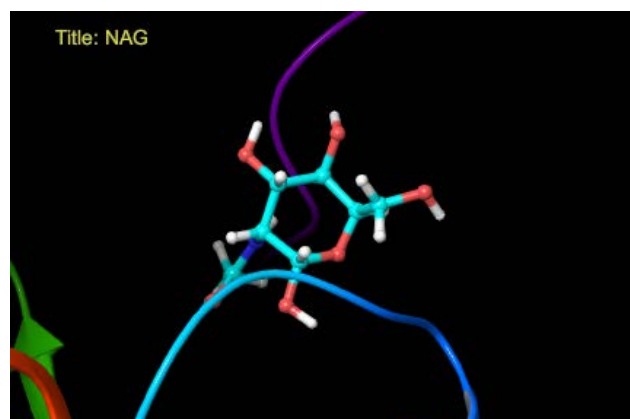
Figure 4. Zoomed in picture of the docked single template ICMV model.

in number in comparison to single template model. Docking of the model built was then performed using NAG and the glide score was found to be -6.755 which proves the latter to be a good inhibitor for the ICMV. Figure 7 shows the picture of the docked chimera model. Several other options were checked from Table 1. But the one giving the best glide score is produced in this work.

Multiple Template (Consensus Model)

Consensus model built is used to identify common structural elements in set of related proteins, which we might want to do at the beginning of a project. This implies that
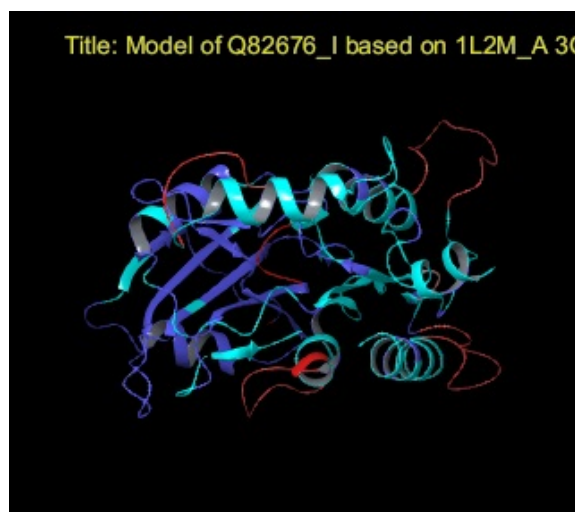
Figure 5. Chimera model of ICMV based on IL2M and 3OIC.
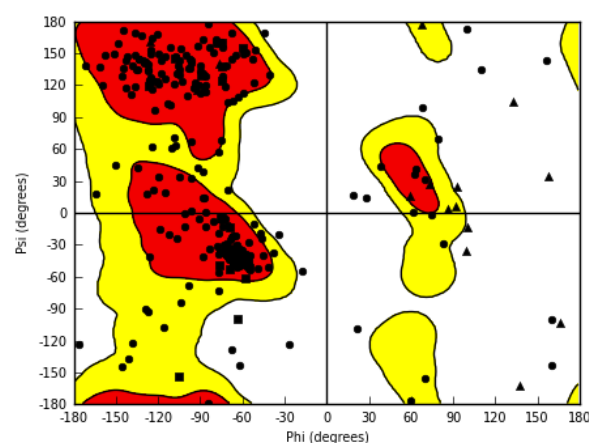
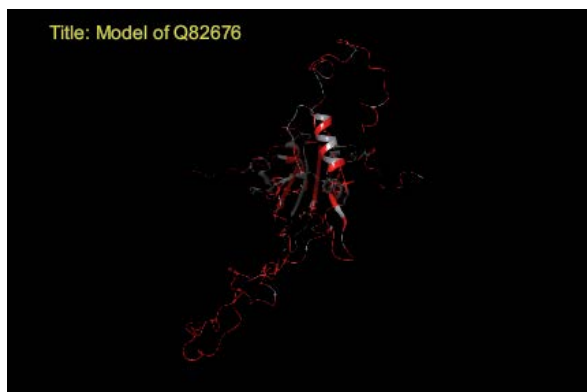**Figure 6.** Ramachandran plot for model built using chimera tools

Figure 7. Chimera model with NAG docked at the most active site.

after importing the proteins we align them structurally and then look for structural entities such as ligands, waters, and counter ions that are in the same region in all or a consensus of the structures. The consensus helps us to identify different binding modes of the ligands, structural waters, and salt bridges.

The entire procedure followed in the previous sections was repeated for the consensus model built using three

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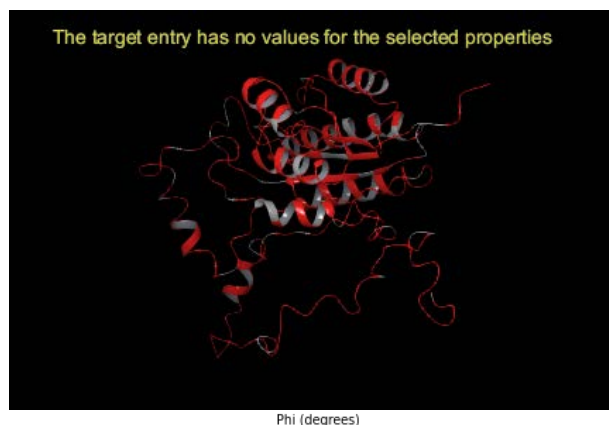J. Integr. Sci. Technol., 2016, 4(2), 81-86   84

templates and greater than three templates. The results are respectively shown in the following figures (Figures 8-11)

**Figure 8.** Consensus model of ICMV using 3 templates.
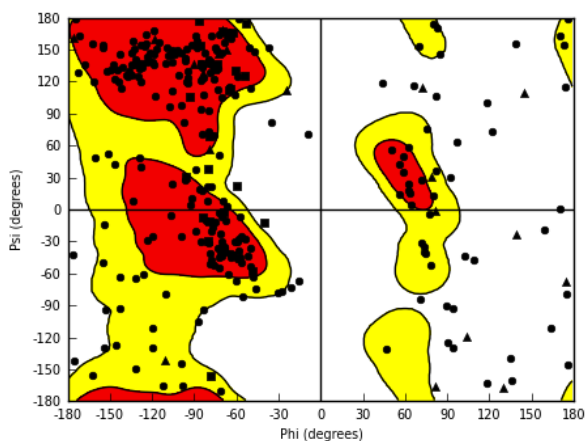
Figure 9. Ramachandran plot for consensus model built using 3 templates

Figure 10. Consensus model of ICMV using >3 templates.

Since the models obtained were <90% accurate, so multiple template model using >3 templates were also tried in which there was improvement marked but it was only marginal to be considered for docking. Docking provided a good glide score but not as efficient as in case of the Chimera model. So, after having worked with the consensus model and its docking, NAG yet again proves to be an effective inhibitor.
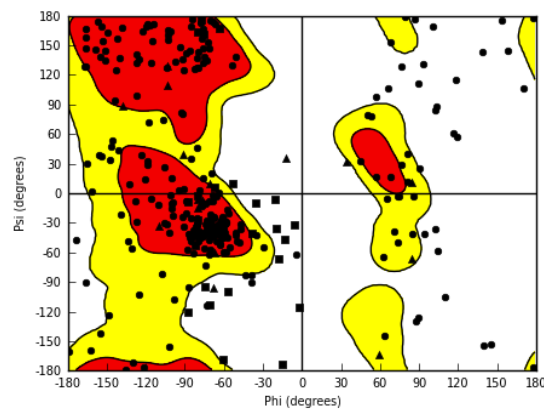
Figure 11. Ramachandran plot of ICMV using >3 templates.

CONCLUSIONS

In this work the three dimensional structures of Replication Association Protein were constructed using the tools of homology modeling successfully. All of them showed an accuracy within the moderate range but the ones obtained using the single template and chimera are found to be more reliable than the consensus models. The latter models experienced a remarkable accuracy of more than 90%.

N-Acetyl D-Glucosamine proves to be an effective inhibitor for docking each of the models and therefore now this work can be taken to the wet lab to try and test the efficiency of the present theoretical work. The aim of docking is to only to check how proteins interact with small chemical molecules and which model serves the best for such studies. The glide score showed the best results in case of chimera model. Since the models accuracy did not vary drastically in each of the cases therefore it is difficult to predict whether single or multiple templates should generally be followed to build a homology model of any query picked up. It may vary depending upon the nature of protein in each case and the kind of homology represented by various templates obtained using blast search.

We hope that the work done in this paper provides sufficient information to start working on the building of the models for Begomoviruses as the 3-D crystal structures are not readily available and the need of the hour is to control its growth lest the situation goes out of hands.

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