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Homology modelling and molecular dynamics study of plant defensin DM-AMP1

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Abstract

Defensin in plants are the most important types of antimicrobial protein that provides the natural immunity against the pathogens. In the present work a stable protein model of plant Defensin DM-AMP1 has been proposed, whose three dimensional structure is not known. The method comprises the homology based modelling of the protein by using MODELLER program and validation of the model by various tools. Molecular dynamics simulation of the model protein was performed in water. The stability of the model was realised by computing root mean square deviation (RMSD) fluctuation of Carbon alpha back bone and potential energy value.

Key words: Defensin, Homology modelling, model validitation, Molecular dynamics simulation

Introduction

Plant Defensins are a prominent family of cationic peptides in the plant kingdom. They are structurally and functionally related to the other Defensins that have been previously studied in other organisms like mammals and insects (Andre de Oliveira Carvalho et al 2009). The overall structure in case of plants comprises of molecular masses between 5 and 7 kDa and possesses a pattern of eight conserved Cys residues. The general architecture shows that it has three antiparallel β-sheets and one α-helix which is stabilized by a structural motif composed of disulfide bridges. This motif is found in other peptides with biological activity and is called the Cys stabilized αβ motif (Jose-Estanyol M et al 2004).

The expression of DM-AMP1 protein suppresses the growth of *M. oryzae* and *R. solani*. The antifungal plant Defensin DmAMP1 interacts with fungal sphingolipids of mannosyl-diinositol phosphorylceramide M (IP) 2C) class and suppresses the fungal growth (Alerts et al. 2006; Thevissen et al. 2000). The presence of potential antifungal characteristics of the protein is suitably to be used as a tool in the agricultural biotechnology sectors.

The three dimensional structure of the Defensin DM-AMP1 is not reported to have been resolved. In the present study, a computational approach is used to predict the three dimensional structure of the DMAMP1 by homology modelling. The approach produces the valid structural model with the available template which having suitable amino acid identity.

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Materials and Methods

Sequence retrieval and 3D model building

The sequence for the Dm-Amp1 peptide was retrieved from SWISSPROT database having ID P0C8Y4 (Rupert W. Osborn, Genoveva et al. 1995). Then with this query sequence a BLAST (Altschul et al. 1990) search was performed against PDB (Protein Databank) to retrieve the corresponding template for the peptide DM- AMP1.The model was built by homology modelling and for this MODELLER (A. Sali, T.L. Blundell 1993) program was used. The MODELLER program uses an automated approach to comparative protein structure modelling by satisfaction of spatial restraints (Eswar N et al. 2008).

Model validation and molecular dynamics study

The MODELLER generated structure was further verified by PROCHECK (Laskowski R A et al. 1998).The PROCHECK program provides the information about the stereo chemical quality of a given protein structure. The PROCHECK was used to generate Ramachandrans plot and the quality of the structure was computed in terms of % of residues in favourable regions, % of non Proline, Glycine residues etc.The quality of structure was also further accessed by using ERRAT (V C. Colovos ,T. O. Yeates 1993). ERRAT is a protein structure verification algorithm that is especially well-suited for evaluating the progress of crystallographic model building and refinement. Along with ERRAT data the DOPE (discrete optimized potential energy) score residues were computed (Min-Yi Shen, Andrej Sali 2006). DOPE score is calculated by Modeller program which indicates the distance dependent statistical potential based on probabilistic theory.

For the calculation of RMSD (Root mean square deviation) and Potential energy value of the model protein GROMACS 4.0.4 (Groingen Machine for Chemical Simulation) tool was used (Hess B, Kutzner C et al. 2008).GROMACS is a versatile package to perform molecular dynamics that simulates the Newtonian equations of motion for systems with hundreds to millions of particles. The model was subjected to molecular dynamics simulation in water at 300 K and 350K temperature and for 1000 pico second by using Gromos 43a1 force field of GROMACS tool. The computing facility utilised is High performance cluster for Biological Applications which is based on Intel Xeon Dual Quad core as processor, Gluster HPC 1.3 X86-64 bit edition ,total 16 nodes each having 4GB of memory.

Results and Discussions

Homology modelling of DM-AMP1

For homology modelling the suitable template structure selected was PDB ID 1BK8 chain A(Fant F, Vranken WF, Borremans FA 1999), which having 68 % identity with the query sequence. Then MODELLER was used to generate the three dimensional structure (Figure 1).

Figure 1. Showing of final 3D structure of the DM-AMP1.Yellow colour shows beta sheets and Red one is the alpha helix

Table 1. The structural information of the DM-AMP1 model.

Structural Features	Information
No. of residues	50
No. of hydrogen bonds	27
No. of atoms	465
No. of helices	1
No. of strands	5
No. of turns	5

Structure validation by ERRAT, DOPE score and PROCHECK tools

To verify further the predicted structures, the coordinates of both predicted structures were fed into the ERRAT Protein Verification Server. The overall quality factor was obtained as 71.429 which are very much satisfactory (Figure 2).The DOPE scores of both template and model obtained from Modeller output. From the comparative chart the peak is showing that there is no defect in the loop regions in the residues. So in the present case the loop refinement method is not required for the model.

Structure validation by PROCHECK

The validation of the model was carried out using Ramachandran plot calculations computed with the PROCHECK program. The Φ and Ψ distributions of the Ramachandran plots of non-Glycine, non-Proline residues are summarized in Figure 4 and Table 2. Altogether

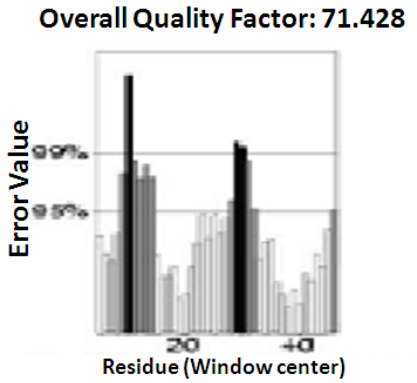

Figure 2. Showing ERRAT structural quality factor

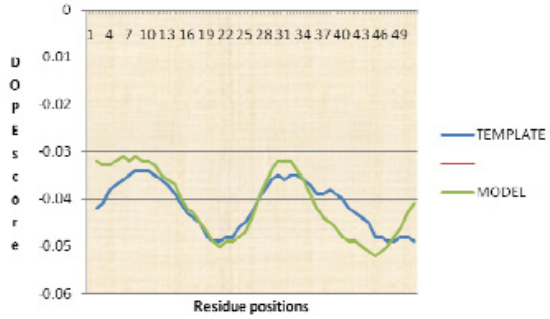

Figure 3. Showing the comparative DOPE value of template and model obtained from MODELLER output

100% of the residues were in favoured and allowed regions. The overall G-factor used was computed as -0.24.

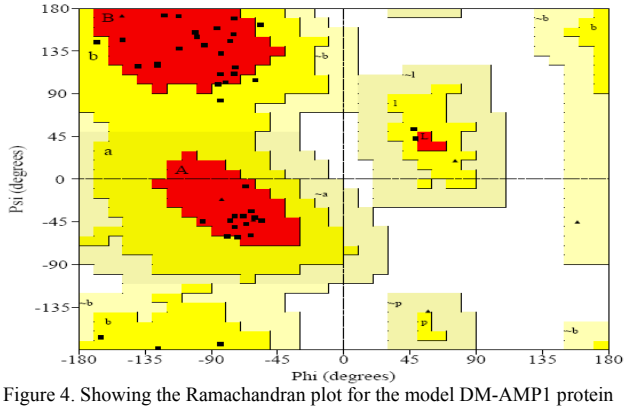

Figure 4. Showing the Ramachandran plot for the model DM-AMP1 protein

Molecular dynamics study

The molecular dynamics simulation was performed in presence of water. Both energy and root mean square deviation plots were derived from the respective trajectory file by Gromacs software output. The RMSD fluctuation plot shows the C-alpha backbone deviation during the simulation process at 300K and 350 K temperature is within the range 0.15-0.45 (Figure 5). The potential energy for the protein was observed during course of simulation is

Table 2. Ramachandran plot calculations on 3D model of DM-AMP1 peptide computed with the PROCHECK program

% residues in favourable regions	91.4
%residues in additional residue regions	8.6
%residues in generously regions	0.0
%residues in disallowed regions	0.0
% of non Proline and non Glycine residues	100

minimum (Figure 6). Since the above results falls within tolerable value hence it confirms the stability of the model protein.

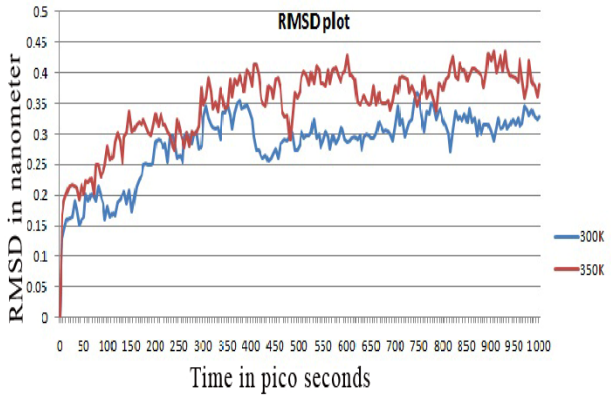

Figure 5. Showing RMSD fluctuation of C- alpha back bone during simulation

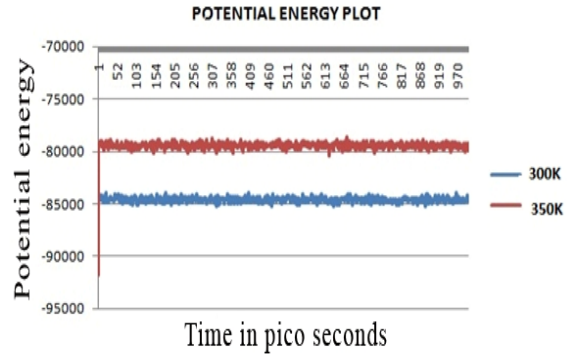

Figure 6. Showing potential energy profile of the protein molecule during molecular dynamics simulation at 300 and 350K

Conclusion

The plant Defensin DM-AMP1 is one of the most potent anti microbial peptide which provides the natural resistance against the pathogenic fungi. In the present work, a homology based 3D model of DMAMP1 peptide has been constructed, using the MODELLER software. The final refined model was further assessed by ERRAT, PROCHECK, Verify 3D programs, and also by molecular dynamics simulation method in water. The results suggest that this model is reliable. The validated protein model proposed in this study may be used further to understand the potential mechanism of antimicrobial property of the protein.

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Contributions

Mr.Raghunath Satpathy was responsible for the designing and conducting the experiment.

Mr.Rashmiranjan Behera was responsible for molecular dynamics simulation data analysis.

Mr. Rajesh Ku. Guru was responsible for homology modeling and also overall editing of the manuscript.

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