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Pectin Methylesterase Estimation in Plant-Tobamovirus Interaction and Comparative Modelling, Active Site Prediction and Docking Between the Proteins Involved in the Movement of Tobamoviruses

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Abstract

Pectin Methylesterase (PME) activity in tobacco and tomato was estimated spectrophotometrically at different time intervals after inoculation with TMV and ToMV respectively. Models of Movement Proteins (MP) of two tobamoviruses (TMV and ToMV) and Pectin methylesterase of tomato were constructed by homology modelling. The program MODELLER was used to build models of each sequence according to the comparative protein modelling method. Model refinements were carried out using the Kollman molecular mechanical force field. Models were analyzed by Ramachandran plot method. Furthermore, the active site prediction in the modeled proteins was carried out by using the CastP server. The complex of MP of TMV- tobacco PME and MP of ToMV- tomato PME was elucidated. Based on this we predicted that, the interaction between pectin methylesterase of hosts and movement proteins of viruses is determined by using the single amino acids (at the active sites) and this determines spread of virus in host plants. The protein complex gives an insight into the interaction between host proteins and viral proteins.

Keywords: Tobamoviruses, Comparative Modeling, Movement protein, Pectin Methylesterase, Active sites, Docking.

Introduction

After initial infection, plant viruses mainly move from cell to cell through plasmodesmata until they reach the vascular system; the viruses are then transported systemically through the vasculature. Presumably, viral spread through the vascular tissue is a passive process, occurring with the flow of photo assimilates (Leisner and Howell, 1993); in contrast, cell-to-cell movement is an active function, requiring specific interaction between the invading virus and plasmodesmata. This interaction is mediated by virus-encoded non-structural movement proteins (MPs) (Lazarowitz and Beachy, 1999).

Numerous viruses have been shown to encode non-structural protein(s), termed the movement protein (MP) that functions in the transport of viral infectious material through plasmodesmata. Plant viruses are classified based on requirement of coat protein and movement protein for movement in the host plant (Table 4. 1). The ability of a virus to move in any given host is determined not so much by compatibility between the genome and the MP as by interactions between the MP and host proteins. Several host proteins have now been identified that interact with MPs; these are categorized in Table 4. 2 according to their (predicted) cellular function or biochemical activity. The several type I MPs interact with transcription factors. Mainly host proteins that interact with MPs are surface-associated proteins, chaperone-like proteins and cytoskeleton proteins (Table 4. 2). Thus, although it appears that different host factors interact with viral MPs, the concept of functional equivalence suggests that most MP interacting host proteins could provide direction for transport towards the cell periphery or even directly to the plasmodesmata (Scholthof, 2005).

Tobacco mosaic virus (TMV) MP, one of the best characterized viral MPs, has been shown to localize to plasmodesmata (Ding et al., 1992), increase plasmodesmal permeability (Waigmann et al., 1994), cooperatively bind single-strand nucleic acids (Citovsky et al., 1992), and interact with cytoskeletal elements (McLean et al., 1995). Based on these observations, MP was proposed to form complexes with the transported genomic TMV RNA, move these complexes throughout the cell using the cytoskeletal network, and target them to and through the enlarged plasmodesmal channels (Ghoshroy et al., 1997).

Pectin, the major component of the plant cell wall plays an important role in functions like cell expansion, growth, and fruit ripening as well as during infection by phytopathogenic microorganisms (Willats et al., 2001). After secretion into the wall as a highly methylesterified form, pectin is de-esterified in muro by pectin methylesterases (PMEs) (E.C. 3.1.1.11) in a spatially regulated manner during development (Knox et al., 1990). Demethylation leads to the formation of polyuronides aggregating into calcium-linked gels that are important in controlling the porosity and mechanical properties of the wall (Willats et al., 2001). PMEs produced by plants take part in important physiological processes (Pilling et al., 2004). They have also been reported to play a role in response to fungal pathogens (Wietholter et al., 2003) and are required for the systemic spread of TMV through the plant (Dorokhov et al., 1999; Chen et al., 2000; Chen and Citovsky, 2003).

Majority of the proteins have sequences but not the structure of the proteins. Recently comparative protein modelling or knowledge based modelling or homology

modelling is being used regularly to elucidate the structure of the proteins, which were not determined by experiments. A homology model based study of Potato leafroll virus (PLRV) (Terradot et al., 2001) and five cucumovirus CPs was previously reported (Gellert et al., 2005). With this background, the present study was carried out to fulfill the following objectives. i). Spectrophotometric quantification of pectin methylesterase in host plants inoculated with plant viruses at different time intervals, and ii). Homology modelling of protein structure, active site predictions and docking between the modelled proteins.

Materials and Methods

Maintanance of host plants, Indicator plants and viruses

The host plants tomato cv. PKM-1 and tobacco (Nicotiana glutinosa) were maintained in an insect-proof screenhouse. TMV was maintained on bell pepper plants and ToMV on tomato plants.

Pectin methylesterase activity

The tomato and N. glutinosa plants were inoculated with ToMV or TMV from pre-maintained tomato and bell pepper seedlings respectively. Inoculation of viruses was done by using 0.1g of virus-infected leaf extracted in 0.1 M phosphate buffer, pH 7.0 by using pre-chilled pestle and mortar. The leaf samples were harvested at different time intervals. The harvested leaf samples were used for the enzyme studies.

One gram of frozen leaf tissue was ground in liquid nitrogen using mortar and pestle and homogenized in 3 ml of 8.8% NaCl (pH 7.55). The homogenate was centrifuged at 20,000g for 10 min at 4^{o}C and the supernatant was retained. The reaction mixture consisted of 2 ml of substrate solution (1% citrus pectin, 0.1 M phosphate buffer of pH 7.5, 5 mM p-nitrophenyl acetate and 100 µl of crude extract). The reaction was carried out for 1 h at 30^{o}C. After incubation, 7 ml of 0.1M phosphate buffer (pH 7.0) was added and samples were read at 400nm. Reaction mixture without substrate was used as control, the extracts boiled for 30 min were used as negative controls. A calibration curve was used with p-nitrophenol as standard. PME activity was expressed as p-nitrophenol produced per unit protein (µmol $mg^{-1}h^{-1}$).

Homology modelling, model refinement, structure evaluation of the movement proteins and pectin methylesterases and PROSITE search

The sequences appear in the EMBL/GenBank/DDBF databases under accession numbers Q7TER6_9VIRU, MOVP_TOMS1, Q9SC79_TOBAC and Q564D7_LYCES for the MP-TMV, MP-ToMV, PME-Tobacco and PME-Tomato, respectively. All the protein models were constructed employing the MODELLER 8v2 program. Molecular graphics and the electrostatic potential representations were created by the Swiss PDB Viewer 3.7 (Guex and Peitsch, 1997). The above-modeled structures were validated using SAVS (http://nihserver.mbi.ucla.edu/SAVS/) and cross-checked with the Ramachandran plot. Active site in modeled protein was predicted by using CASTp (Binkowski et al., 2003) for all the proteins. The formation of protein complex was studied by using HEX 4.1(Ritchie, 2003).

Statistical analysis

The data generated was average of three independent experiments. Data was subjected to analysis of variance (ANOVA) and the means were compared for significance using Duncan's Multiple Range Test (DMRT, P=0.05)

Results

PME activity in tobacco plants inoculated with TMV

The Nicotiana glutinosa plants inoculated with TMV showed an increase in the PME activity in early hours of virus inoculation in comparison with buffer-inoculated (control) plants. Maximum PME activity was noticed at 12 hpi, this activity was 15 fold more in comparison with control. From 24 hpi the enzyme activity was declined.

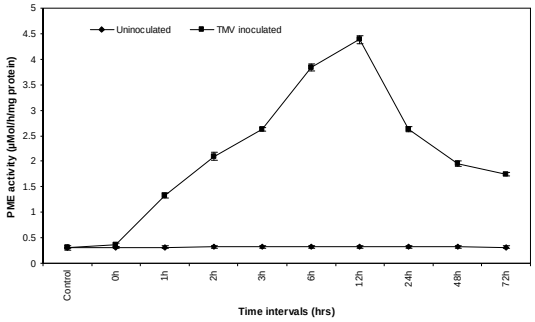

Figure 1: Time course study of PME activity in tobacco (Nicotiana glutinosa) inoculated with Tobacco mosaic virus. The results are the mean of three replicates ±SE of means.

PME activity in tomato plants inoculated with ToMV

The ToMV-inoculation to tomato seedling showed a steady increase in the PME activity in virus-inoculated ones in comparison with buffer-inoculated (control) plants. The maximum PME activity was noticed at 48 hpi, 20-fold increased, in comparison with control. The decline in the enzyme activity was noticed at 72 hpi.

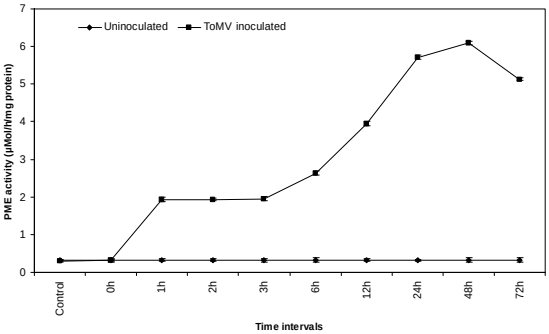

Figure 2: Time course study of PME activity in tomato inoculated with Tomato mosaic virus. The results are the mean of three replicates ±SE of means.

Homology modelling, model refinement, structure evaluation of the Movement proteins and pectin methylesterases and PROSITE search

The initial model of pectin methylesterase of tomato along with movement proteins of tobamoviruses (TMV and ToMV) were built by using homology-modeling methods and the MODELLER software (Sali et al., 1993). The query sequences were searched to find out the related protein structure to be used as a template by the BLAST (Basic Local Alignment Search Tool) program against PDB (Protein Databank). Sequences that showed maximum identity with high score and less E-value were aligned and were used as a reference structure to build a 3D model for proteins. The co-ordinates for the structurally conserved regions (SCRs) for all the proteins were assigned from the template using multiple sequence alignment, based on the Needleman-Wunsch algorithm. The final structure obtained was analyzed by Ramachandran's map using PROCHECK, a program to check the stereochemical quality of protein structures, and environment profile using VERIFY-3D graph (structure evaluation server). This obtained model was used for the identification of active site and for docking of the substrate with the enzyme. The templates and methods used to construct the models for proteins used in our study was summarized in the table 1.

Table 1: Summary of proteins used for protein modeling, methods used for modeling.

Protein	Template	Homology (Identity) (%)	Method used
Movement protein of TMV (Figure 3)	1ITK_B	34	Homology modeling
Movement protein of ToMV (Figure 4)	1FP9	31	Fold recognition
PME of tobacco (Figure 5)	1GQ8	X-ray structure	-
PME of tomato (Figure 6)	1GQ8	83	Homology modeling

Ramachandran plot studies for the modelled protein of movement protein of TMV showed 80.2% amino acid residues in core region where as only 0.8% of residues in disallowed region. In a similar way, Ramachandran plot showed 81.5% residues in core region and 1.7% in disallowed region for ToMV MP, 86.8% residues in core region and importantly no residues in disallowed region for Tobacco PME and 86.9% residues in core region and 1.1% residues in disallowed region for Tomato PME.

Model-based identification of amino acid residues

Active site prediction by using castP server shows that the modeled proteins have a single amino acid residue that acts as an active site, which has an important biological role. Binding sites and active sites of proteins and DNAs are associated with structural pockets and cavities. castP server uses the weighted Delaunay triangulation and the alpha complex for shape measurements. It provides identification and measurements

of surface accessible pockets as well as interior inaccessible cavities, for proteins and other molecules. It measures analytically the area and volume of each pocket and cavity, both in solvent accessible surface (SA, Richards' surface) and molecular surface (MS, Connolly's surface). It also measures the number of mouth openings, area of the openings, and circumference of mouth lips, in both SA and MS surfaces for each pocket.

Movement proteins of TMV and ToMV had ARG 85 and LYS 60 as active sites, present in the cavities respectively of modelled proteins when subjected to castP Server. In the similar way, the model of Pectin methylesterases of tobacco and tomato has showed active sites as TYR 230 and SER 540 respectively (Fig 7-10).

Docking studies

The interactions studies carried out by using HEX 4.1 and Z-dock server showed that the active site residues present in movement proteins of TMV and ToMV are interacting with active site residues of PME of tobacco and tomato respectively. The interaction between the proteins is due to the formation of hydrogen bond between proteins (Active sites) of MPs encoded by viruses and PMEs of host plants (Fig 11-12).

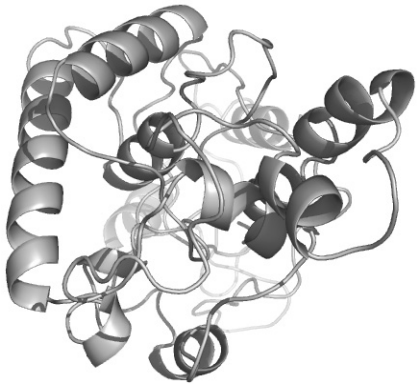

Figure 3: Ribbon representation of movement protein of TMV. (PDB ID: 2IP5).

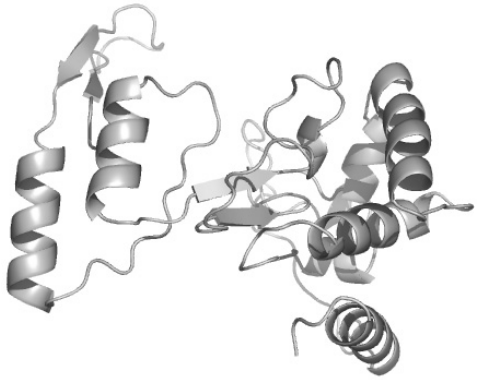

Figure 4: Ribbon representation of Movement protein of ToMV. (PDB ID: 2IP8).

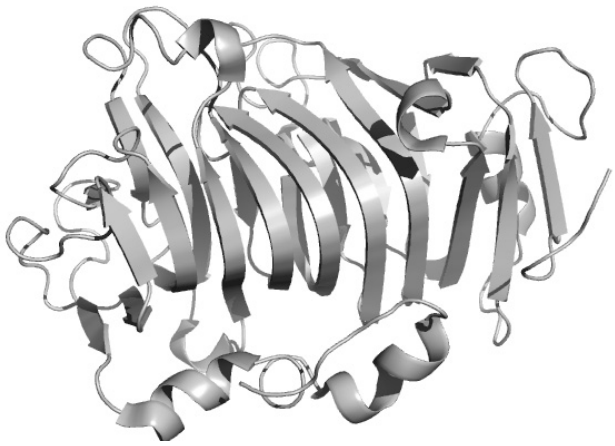

Figure 5: Ribbon representation of Pectin methyl esterase of tobacco.

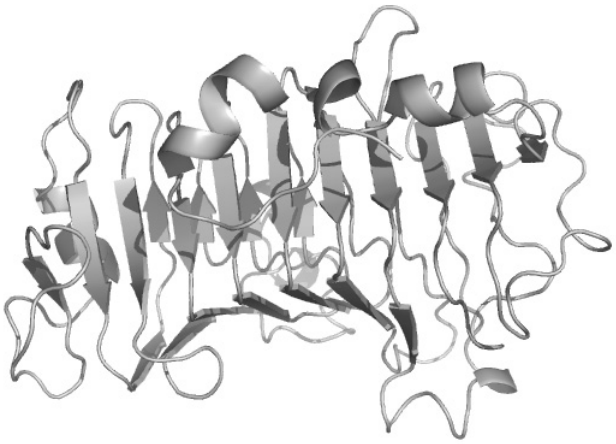

Figure 6: Ribbon representation of pectin methyl esterase of tomato.

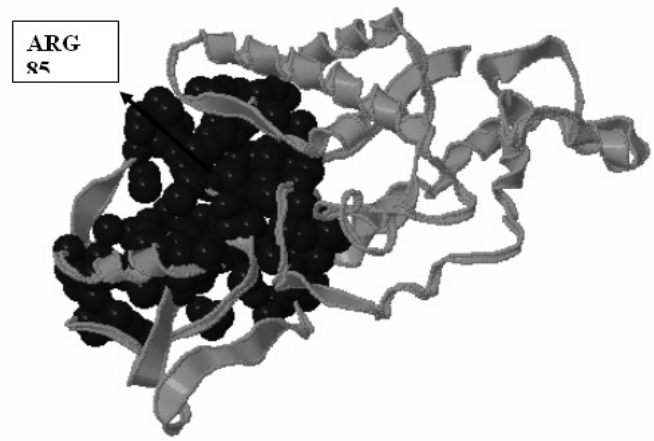

Figure 7: Active site prediction for Movement Protein of TMV. (Interacting residue Arg85 is located in this cavity).

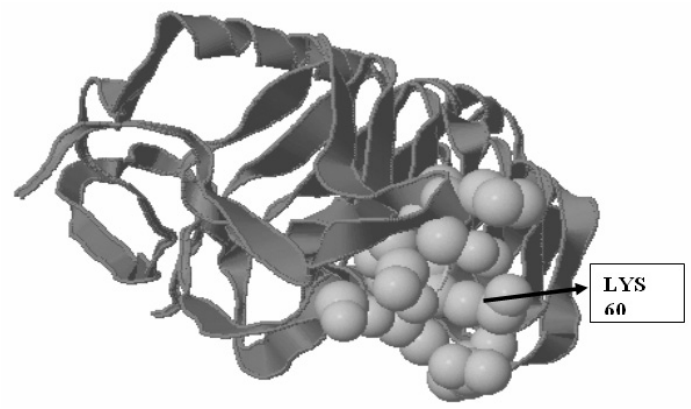

Figure 8: Active site prediction for Movement Protein of ToMV. (Interacting Residue Lys60 is located in this cavity).

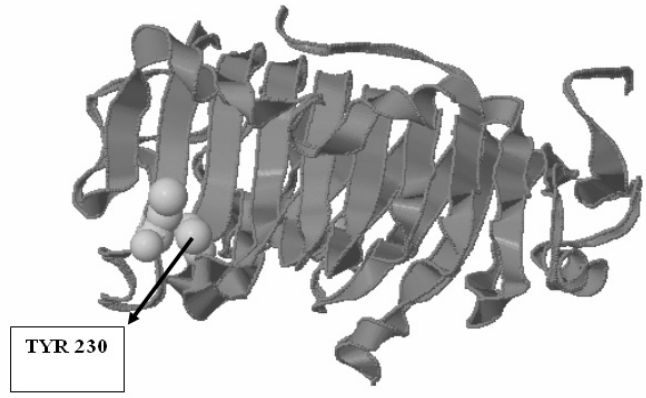

Figure 9: Active site prediction for Pectin methylesterase of Tobacco. (Interacting residue Tyr230 present in this cavity).

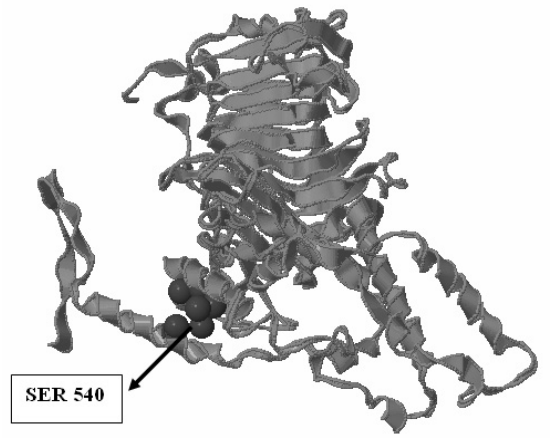

Figure 10: Active site prediction for Pectin methylesterase of Tomato. (Residue in this area is Ser 540).

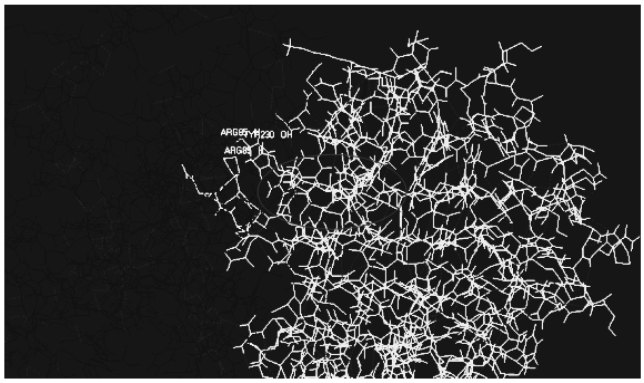

Figure 11: Labels showing the H-bond interaction between the residue Tyr230 of Pectin methylesterase of tobacco and residue Arg85 of movement protein TMV (both the interacted residues are active site residues and present in cavities, when analyzed using by CASTp server).

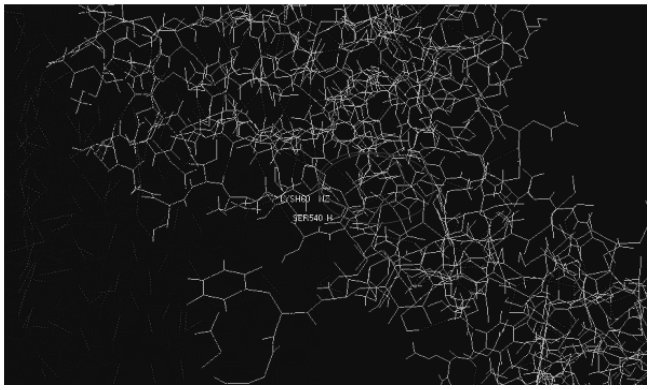

Figure 12: Labels showing the H-bond interaction between the residue Ser540 of Pectin methylesterase of tomato and residue Lys60 of movement protein of ToMV (both the interacted residues are active site residues and present in cavities, when analyzed using by CASTp server).

Discussion

Protein-protein interaction plays an important role in the movement of the biomolecules in the plant systems. The viral movement in the host plant requires several protein interactions. Interactions between tobamovirus proteins and host factors have been reported from a variety of plants, ranging from resistance gene products to ubiquitous host gene products that un-doubtably more than one viral group has used to their advantage. MP binds to the cell wall-associated protein pectin methylesterase (Dorokhov et al., 1999; Chen et al., 2000). The MP has also been found to associate with microtubules, which may play a role in trafficking viral RNA within the cell (Boyko et al., 2000).

The role of pectin methylesterase role is explained by several researchers in various fields in plant systems including its role in virus movement. Reports reveal that, PME binds with the movement proteins of tobamoviruses along with other proteins like AtP8 and TIPs (Scholthof, 2005). The reduction in levels of PME resulted in delay in viral systemic spread. The movement of virus was slowed down but not blocked (Chen and Citovasky, 2003). In our studies, the gradual increase in PME upto certain time intervals followed by gradual decline in the enzyme activity in Nicotiana glutinosa-TMV interaction may be attributed to the appearance of pin head sized local lesions at the point of infection but in tomato-ToMV interaction the gradual increase in the PME activity may be due to the compatible host nature of tomato to ToMV infection.

In the absence of an experimentally determined protein structure, comparative modeling may provide a structural platform for the investigation of sequence-structure-function relationships. This technique requires a homologous template structure to be identified and the sequence of the modeled protein (a target) to be correctly aligned to the template. Each protein has an active site to which the other proteins bind and interact with each other, which is an important step in the biological molecule trafficking in the plant tissues. Recently Gellert et al. (2005) constructed models for five cucumovirus coat proteins by using homology modelling methods and they also carried out the protein structure based functional analysis of coat proteins. In present study, homology models for the movement proteins of tobamoviruses (TMV and ToMV) were constructed along with PME of tomato by using homology modelling methods. Thus, modeling of proteins of both viral origin and plants was carried out to know the structure, active sites and the interaction between the proteins and the interacting molecules, which play an important role in biological activity (Tkaczuk et al., 2006). In our study, the models for the movement proteins of TMV and ToMV along with PMEs of tobacco and tomato were carried out by using the Modeller. Similarly, Gellert et al. (2005) carried out the homology modelling and protein structure based functional analysis of five cucumovirus coat proteins.

In this work, we have constructed a 3D model of Pectin methylesterase enzyme from Tomato along with movement proteins of TMV and ToMV, using the MODELLER software and obtained a refined model after energy minimization. The final refined model was further assessed by VERIFY-3D and PROCHECK program, and the results show that the obtained models were reliable. The stable structure is further used for studying the interaction between proteins (Docking). Docking results indicate that amino-acid residues present (active site) in the modelled proteins play an important role in maintaining a functional conformation and are directly involved in interaction. The interaction between the PMEs and the MPs proposed in this study are useful for understanding the potential mechanism of enzyme and the MP binding during the virus movement. As is well known, hydrogen bonds play an important role for the structure and function of biological molecules, especially for the enzyme catalysis. In this study it was found that amino acid residues present in the active sites of proteins are important for strong hydrogen bonding interaction. Similar results were obtained by Sekhar et al. (2006), studying the hydrogen peroxide binding to catalase in rice.

Further studies can be carried out to understand the movement of the virus in the host plants by constructing models of different proteins of viral origin as well as host origin, which gives the scientists the clear image of the interaction between proteins. In this chapter, the interaction between the pectinmethylesterase and movement protein of tobamoviruses was studied. Furthermore, it is worth while to identify the host factors or proteins participating in the virus movement. Based on the results obtained here, well targeted molecular biological experiments can be planned on the basis of this work.

References

[1] Binkowski, A.T., S. Naghibzadeg, J. Liang, 2003. CASTp: computed atlas of surface topography of proteins. Nucleic Acid Research, 31:3352-3355.

[2] Chen, M.H., J. Sheng, G. Hind, A.K. Handa, V. Citovsky, 2000. Interaction between the tobacco mosaic virus movement protein and host cell pectin methylesterases is required for viral cell-to-cell movement. EMBO Journal, 19:913–920.

[3] Chen, M., V. Citovsky, 2003. Systemic movement of tobamovirus requires host cell pectinmethylesterase. The Plant Journal, 35:386-392.

[4] Citovsky, V., M.L. Wong, A. Shaw, B.V.V. Prasad, P. Zambryski, 1992. Visualization and characterization of tobacco mosaic virus movement protein binding to single-stranded nucleic acids. Plant Cell, 4:397–411.

[5] Ding, B., J.S. Haudenshield, R.J. Hull, S. Wolf, R.N. Beachy, W.J. Lucas, 1992. Secondary plasmodesmata are specific sites of localization of the tobacco mosaic virus movement protein in transgenic tobacco plants. Plant Cell, 4:915–928.

[6] Dorokhov, Y.L., K. Makinen, O.Y. Frolova, A. Merits, J. Saarinen, N. Kalkkinen, J.G. Atabekov, M. Saarma, 1999. A novel function for a ubiquitous plant enzyme pectin methylesterase: The host-cell receptor for the tobacco mosaic virus movement protein. FEBS Letters, 461:223–228.

[7] Gellert, A.K., K. Salanki, G. Naray-Szabo, E. Balazs, 2005. Homology modelling and protein structure based functional analysis of five cucumovirus coat proteins. Journal of Molecular Graphics and Modelling, 24:319–327.

[8] Ghoshroy, S., R. Lartey, J. Sheng, V. Citovsky, 1997. Transport of proteins and nucleic acids through plasmodesmata. Annual Review Plant Physiology and Plant Molecular Biology, 48:27–49.

[9] Guex, N., M.C. Peitsch, 1997. SWISS-MODEL and the Swiss-PdbViewer: An environment for comparative protein modeling. Electrophoresis, 18:2714-2723.

[10] Knox, J.P., P.J. Linstead, J. King, C. Cooper, K. Roberts, 1990. Pectin esterification is spatially regulated both within cell walls and between developing tissues of root apices. Planta, 181:512–521.

[11] Lazarowitz, S.G., R.N. Beachy, 1999. Viral Movement Proteins as Probes for Intracellular and Intercellular Trafficking in Plants. The Plant Cell, 11:535–548.

[12] Leisner, S.M., S.H. Howell, 1993. Long-distance movement of plant viruses. Trends in Microbiology, 1:314-317.

[13] McLean, B.G., J. Zupan, P. Zambryski, 1995. Tobacco mosaic virus movement protein associates with the cytoskeleton in tobacco cells. Plant Cell, 7:2101–2114.

[14] Pilling, J., L. Willmitzer, H. Bucking, J. Fisahn, 2004. Inhibition of a ubiquitously expressed pectin methyl esterase in Solanum tuberosum L. affects plant growth, leaf growth polarity, and ion partitioning. Planta, 219:32–40.

[15] Ritchie, D.W. 2003. Evaluation of protein docking predictions using Hex 3.1 in CAPRI rounds 1 and 2. Proteins, 52(1):98-106.

[16] Sali, A., T.L. Blundell, 1993. Comparative protein modelling by satisfaction of spatial restraints. Journal of Molecular Biology, 234:779-815.

[17] Scholthof, H.B. 2005. Plant virus transport: motions of functional equivalence. Trends in Plant Science, 10(8):376-382.

[18] Sekhar, P.N., P.B.K. Kishor, L.A. Reddy, P. Mondal, A.K. Dash, M. Kar, S. Mohanty, S.C. Sabat, 2006. In silico modeling and hydrogen peroxide binding study of rice catalase. In Silico Biology, 6: 0041.

[19] Terradot, L., M. Souchet, D.G. Ducray-Bourdin, 2001. Analysis of three-dimensional structure of Potato leafroll virus coat protein obtained by homology modelling, Virology, 286:72–82.

[20] Tkaczuk, K.L., A. Obarska, J.M. Bujnicki, 2006. Molecular phylogenetics and comparative modeling of HEN1, a methyltransferase involved in plant microRNA biogenesis. BMC Evolutionary Biology, 6:6.

[21] Waigmann, E., W. Lucas, V. Citovsky, P. Zambryski, 1994. Direct functional assay for tobacco mosaic virus cell-to-cell movement protein and identification of a domain involved in increasing plasmodesmal permeability. Proceedings of National Academy of Sciences. USA., 91:1433–1437.

[22] Wietholter, N., B. Graessner, M. Mierau, A.J. Mort, B.M. Moerschbacher, 2003. Differences in the methyl ester distribution of homogalacturonans from near-isogenic wheat lines resistant and susceptible to the wheat stem rust fungus. Molecular Plant Microbe Interaction, 16:945–952.

[23] Willats, W.G., C. Orfila, G. Limberg, H.C. Buchholt, V.G.J. Alebeek, A.G. Voragen, S.E. Marcus, T.M. Christensen, J.D. Mikkelsen, B.S. Murray, J.P. Knox, 2001. Modulation of the degree and pattern of methyl esterification of pectic homogalacturonan in plant cell walls. Implications for pectin methyl esterase action, matrix properties and cell adhesion. Journal of Biological Chemistry, 276:19404–19413.