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In-Silico Analysis of Grapevine Germin like Protein (VvGLP3) and its Probable Role in Defense against Powdery Mildew Disease

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Abstract

Germin like proteins (GLPs) is heterogeneous groups of proteins that are part of cupin superfamily. Various GLPs of Grapevine (VvGLP3) have been identified and characterized at molecular, not at structural level. This study analyzes the structure of VvGLP3 to elucidate its role in defense against powdery mildew disease caused by *Erysiphe necator*. Physiochemical analysis through high aliphatic index (AI), low instability index and grand average of hydropathy (GRAVY) reveal that VvGLP3 is heat stable hydrophobic protein. InterProScan was used to check the similarity of VvGLP3 to other GLPs and TMHMM indicates that VvGLP3 is a transmembrane protein. The secondary structure of VvGLP3 reveals that it consists of random coils followed by extended α-helix and β-trends and I-TASSER was employed to construct 3-D structure of VvGLP3. A molecular docking approach was applied to study molecular interaction and binding pattern by Auto Dock Vina. I-TASSER and SOPMA were used to determine the functionally important residues and their role. In the present study molecular dock binding affinity energy is from -1.5 to -1.3 of germin like protein with manganese (Mn^{+2}) co-factor which depicts its role in defense against powdery mildew disease.

Keywords: Germin like protein; powdery mildew disease; molecular interaction; enzymatic properties; Grapevine

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INTRODUCTION

Germin was first recognized in wheat's embryo, as a specific molecule for germination (1). It was later characterized homo-hexamer glycoprotein processing oxalate oxidase (OXO) activity. The molecular form of German like proteins (GLPs) is similar to other proteins (2). Moreover, GLP's are large protein family that has more than twenty-five members. Unlike the Germins (GER), GLPs are found in many of both angiosperms and gymnosperms. Most Germins are non-covalently attached to the extracellular matrix which shows expression in different parts of the plant at different developmental stages of the plants (3). Germins and GLP are the part of cupin superfamily that have conserved beta barrier core, while GLP show enzymatic and non-enzymatic function due to change in the region of active site (4,5). Phylogenetically germin and GLPs are divided into five subgroups (families). These are (1) True GER, (2) Gymnosperm GLPs, (3) GLP subfamily-1, (4) GLP subfamily-2, (5) GLP subfamily-3 (6).

GLPs exhibit six different enzymatic activities based on their roles in different processes. One of these activities is oxalate oxidase activity that is related only to true GER (7). Subfamilies1 and 2 exhibits superoxide dismutase (SOD) activity (8). ADP glucose pyrophosphatase (AGPase) is a specific activity related to subfamily 3 (9) and serine protease inhibition activity is present in wheat's apoplast for defense (10). Similarly polyphenol oxidase (ppo) activity is found in satsesma mandarin (11). Recently, a novel activity called cysteine peptidase has been found in *Thevetia peruviana* (5). Besides, this GLP also plays fundamental role in protein-protein interaction.

Superoxide dismutase (SOD) belongs to the family which catalysis the disproportionation of superoxide anion radical to generate oxygen and water (13). The main role of SOD is to prevent the oxidation of biological molecules by patrolling the radicals that are generated in physiological processes. SOD is divided into three types based on their metal ions. (1) Cu/ZnSOD (located in chloroplast or cytosol of a plant), (2) MnSOD (found in mitochondria) and (3) FeSOD (present in chloroplast) (14). Moreover, SOD is the most enzymatic activity GER and so far it has discovered that only wheat GER possesses both SOD and OXO activity (15). Mainly SOD is associated with the production of H_2O_2 which serves as a signaling molecule to help the defense mechanism (16). Among the seven discovered GLPs grapevine, only VvGLP3 exhibits SOD activity that is localized to cell wall and aid in defense against powdery mildew disease (17).

Powdery mildew disease is one of the economically important diseases caused by fungus *Erysiphe necator*. *Vitis vinifera* is most commonly cultivated species of grapevine and highly susceptible to this disease. Powdery mildew disease causes many economic losses due to cost of fungicide application and loss of grape production. But the emerging resistance to fungicides brings the need to develop new molecular strategies for defense against fungal disease (18). Powdery mildew defense is attained in several ways including papillae formation at the site of penetration, accumulation of GER related to pathogenesis and the death of attached hypersensitive epidermal cells. These proteins build a barrier against the pathogen by antimicrobial and antifungal activities (19). N-acetylglucosamine (GlcNAc) is the monomer of the polysaccharide chitin and is an essential structural component of the fungal cell wall and the arthropod exoskeleton.

It was reported that *V. vinifera* has 7 GLP members i.e. VvGLP1 to VvGLP 7. It was reported that one of the grapevine GLP genes, VvGLP3 is induced in response to powdery mildew disease in epidermal cells. VvGLP3 was determined as subcellular localized protein by exhibiting green fluorescent protein GFP fusion construct in onion cells using transgenic approaches. VvGLP3 provides defense against *E. necator* by exhibiting SOD activity. Several studies have been done on different aspects of GLP of grapevine but no work has related to VvGLP3 protein structural analysis. This study is performed on VvGLP3 protein, to identify its interaction with other metal ion and analyze its role in defense by molecular docking.

Materials and Methods

Physiochemical properties

To determine the physiochemical characterization, ExPASy's Protparam tool was used. The basic properties were determined based on molecular weight, instability index, isoelectric point, aliphatic index and Grand Average of Hydrophobicity (GRAVY) (20). To locate the conserved domains of VvGLP3, InterProScan was used. TMHMM was used to identify the localization of VvGLP3 (21, 22).

Prediction of VvGLP3 3D structure

For the structural validation and prediction of 3-D structure, I-TASSER (Iterative Threading Assembly Refinement), SOPMA and PSIPRED were used (23, 24). The 3D structure of VvGLP3 was built using I-TASSER, probable templates with the PDB IDs; 2ET7, 5WPW and 10D5.

Molecular docking studies

Molecular docking was performed to justify the role of VvGLP3 in defense against a fungi *E. necator*. Docking was performed by AutoDock Vina. For this purpose, the N- acetyl glucosamine was structurally analyzed by using the software. In the docking the receptor protein was kept as rigid molecule and ligands remained flexible. Non-interacting water molecules were excluded from the structure. Manganese (Mn^{+2}) metal ion was used as ligand for this purpose.

RESULTS

Physiochemical properties of VvGLP3

Various physiochemical properties of VvGLP3 were predicted using an ExPasy-Protparam tool. It has computed many of the parameters (Table 1). Protein had a sequence of 225 amino acids. The determined molecular weight is 24 KD. It also provides several other parameters like atomic composition and number of atoms present in total. Extinction co-efficient presumes less absorbance (i.e. 0.765) when there were pairs of cysteine present as compared to when there will be no Cys residues (i.e. 0.759). Instability index (26.44) and aliphatic index (102.18) predict VvGLP3 protein is thermostable. GRAVY prognosis the hydropathy of GLP which indicated its more interaction with water molecules and supports the assumptions about the localization of VvGLP3 protein in trans-membrane.

InterProScan foretells that VvGLP3 protein has been homogenous to other GLP's and Germin showed features that match with cupin

family. The matching views of cupin domains and sites where a Manganese (Mn^{+2}) metal ion binds with it (Fig. 1).

VvGLP3 - A transmembrane protein

Cellular localization was predicted by Transmembrane hidden Markov model (TMHMM) which was a tool that retrieved cellular membrane-embedded and its amino acid path. The results of TMHMM analyze which portion of protein was localized in the cytoplasm, membrane or external to plasma membrane. So, when TMHMM applied to VvGLP3, it results enlisted the protein length, number of predicted TMH's, number of amino acids in TMH's, the no. of first sixty amino acids and lastly the total number of nitrogen in VvGLP3. As estimated by the graph, the probability of presence of VvGLP3 external to cellular membrane was very high whereas it was more likely to cross the transmembrane as indicated by the red vertical lines beneath the curve.

Table I.- Protparam prediction of physiochemical properties of VvGLP3.

Parameters	VvGLP3
Sequence length	225
Molecular weight	24294.95
Extinction co-effecients (with cysteine pairs)	0.765
Extinction co-effecients (no Cys residues)	0.759
Instability index	26.44
Aliphatic index	102.18
GRAVY	0.247

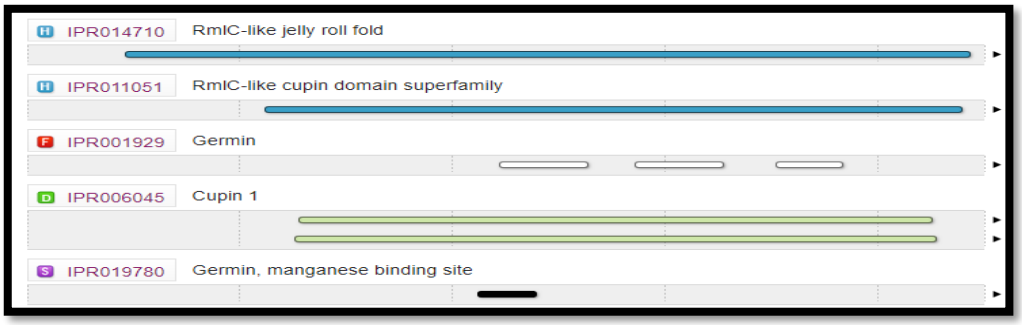

Fig. 1. InterProScan server results showed the homologous superfamily and cupin domain (in green color) and manganese binding site (in black color).

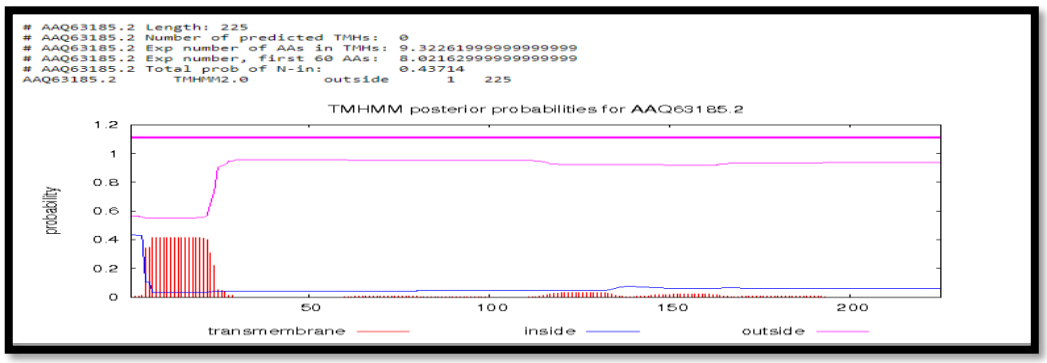

Fig. 2. The position of amino acids that were numbered from N-terminal to C-terminal is shown on the x-axis while the probability of localization in a particular location was given on the y-axis. Vertical red lines represent a portion of that enters or crossed a membrane and blue lines represent a probability of protein in a cytoplasm whereas pink line represents the probability of protein that was present outside the plasma membrane.

Secondary structure of VvGLP3

Three tools were used for the secondary structure prediction of VvGLP3 such as SOPMA, I-TASSER, and PSIPRED protein structure prediction server. Among all predicted secondary structures random coil (40%) were highly abundant. The remaining secondary structure is presented by extended strand (27.11%), alpha-helix (22.67%) and beta-turn (10.22%). The sequence length of amino acid was 225. Default parameters were used for the secondary structure prediction (similarity threshold: 8, window width: 17 and number of states: 4).

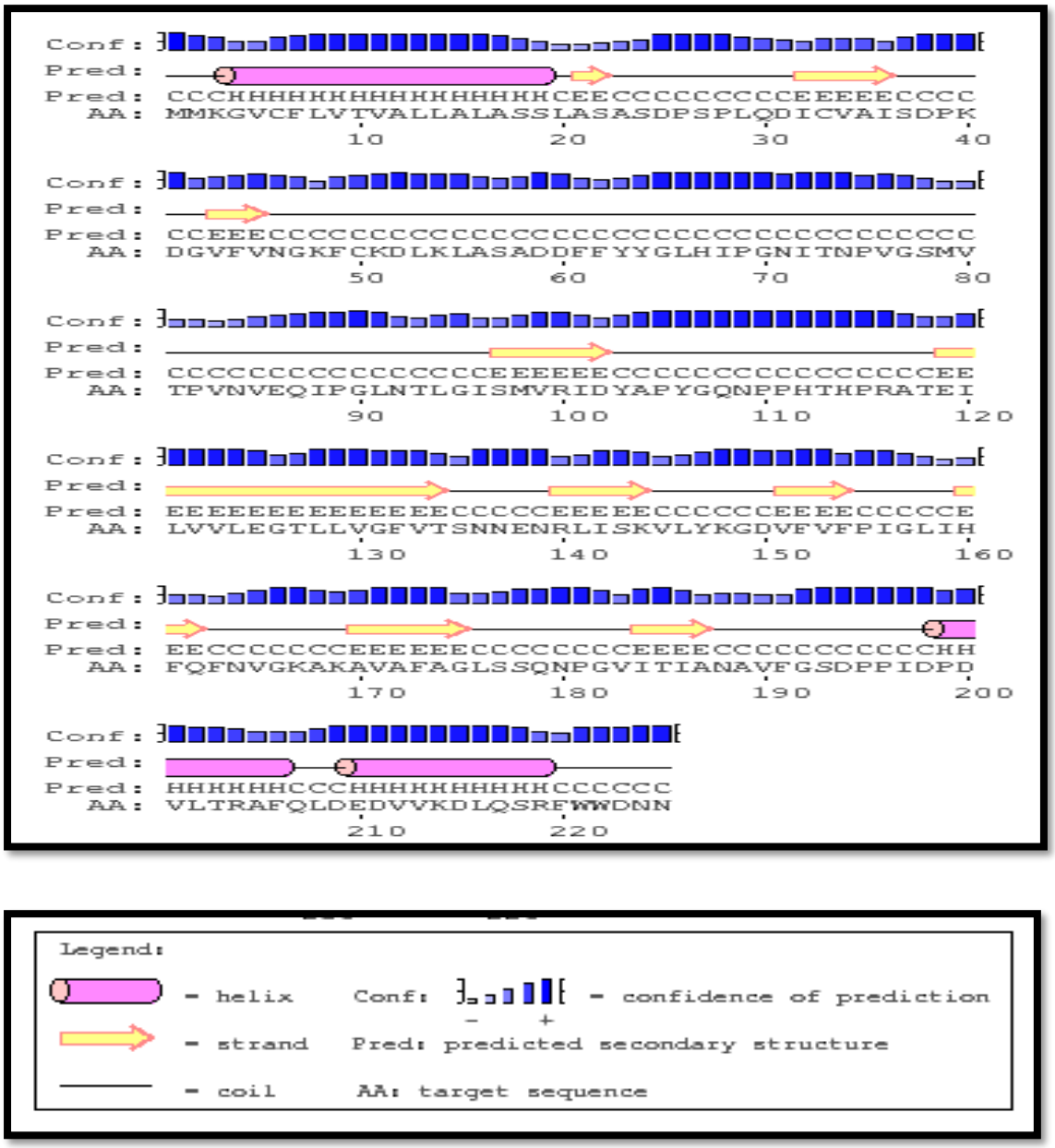

Fig. 3. Results of PSIPRED and I-TASSER also show that random coils are highly abundant in the secondary structure of VvGLP3 in comparison to helix and strand.

Functional key residues
Ribbon presentation of the predicted 3-D structure

Fig. 4. Ribbon view of 3-D predicted structure (A)**,** Pymol used to view top-ranked residue which involved in binding with Mn^{+2} (B), Molecular docking hydrophobic interaction (C).

of VvGLP3 is shown in Fig 3. Residues that are adjacent to carboxyl terminus were colored red and residues adjacent to amino terminus are colored blue. Residues that were between N- and C-terminus are colored across visible spectrum. On three-dimensional structure of VvGLP3 top-ranked residues were shown in red color.

Table II.- Binding affinity of protein obtained as a result of molecular docking.

Predicted functions of VvGLP3

VvGLP3 function prediction was done by using InterProScan and Pfam. Mainly there were two molecular functions performed by VvGLP3 as indicated by InterProScan results. The first function was the manganese metal ion (Mn^{+2}) binding, which shows selective interaction and non-covalent bonding with manganese ion. The second function was the nutrient reservoir activity, shows that it aids in the nutritious substrates storage. Pfam showed that VvGLP3 is functionally similar to cupin 1 family.

Molecular docking

Molecular docking was performed by Vina in which we used protein N-acetyl glucosamine which was the main component of chitin along with the manganese cation which acts as a ligand. During docking procedure, 6 torsion angles were used to rotate the ligand. By applying manual gridding 3 centers x,y,z were set as 7.12, 14.5 and 18.6 respectively. Three dimensions x,y,z were set as 26. Corresponding to the receptor structure each grid was positioned at the midpoint. Docking protocol comprises more than 100 runs and it has maximum number of 87,2992592 iterations. RMSD was the measure of distance between atoms of super-imposed protein. Our analysis showed that the protein has no RMSD tolerance. When the docking completes, it resulted in conformation and specified orientations were analyzed. In order to understand the binding pattern and interacting residues. It was shown that N-acetyl glucosamine has affinity of -1.5Kcal/mol. It was indicated that as there is no RMSD value and have very low affinity so it showed that the protein had very less binding affinity.

DISCUSSION

Physiochemical, functional and structural properties of VvGLP3 were illustrated by *insilico* analysis. VvGLP3 was a hydrophobic protein as indicated by the physiochemical characters and it

was supposed to reside in the extracellular matri. The thermostable property of VvGPL3 was manifested by low instability and high aliphatic Index. Over the previous years, various studies had been done on the grapevine Germin-Like Protein (VvGLP3). One study demonstrates that VvGPL3 accumulates in the region where there is powdery mildew disease as compared to the whole plant. Therefore, it suggests that the expression of VvGLP3 is penetrated by E. necator and is associated only with the epidermal cells (17). Other studies showed that during E. necator penetration in response to the release of oligo-glactoronides, VvGLP3 is induced. These oligo-glactoronides provides defense in a large range of plants, including grapevine (25). The results of InterProScan server reveals the cupin domain and Mn+2 binding site in VvGPL3 and Cupin as a superfamily. The 3D structure of VvGLP3 was also estimated by the use of online server I-TASSER. Protein molecular modeling was performed by Vina and the defense mechanism of protein was also predicted. Grapevine Germin likes protein play role in defense against powdery mildew disease caused by E. necator, as successful colonization of this fungus formed as a result of penetration. It was studied that the synthesis and induction of VvGLP3 can inhibit the penetration of fungus in the selected epidermal cells (26). It was noted that this inhibitory response is too slow to compete the disease. This indication was supported by other researchers whose work shows that barley Germin HvGER4 was abundant in the cells which are affected by the disease (27). Further insilico analysis is required to determine the factors that enhance the VvGLP3 response in susceptible cells with the aim of enhancing the response. This study is helpful for protein structure and function prediction. It also gives perception of variety of enzymatic activities in cupin superfamily.

Conclusion

The study demonstrates that VvGLP3 is Germin like protein found in the grapevine. This VvGLP3 is induced in response to powdery mildew disease which is caused by *E. necator*. This protein is targeted to cell wall of infected epidermal cells and is capable of acting as a source of hydrogen peroxide (H_2O_2) as it retains SOD activity. The defense is attained by cell wall reinforcement in response to pathogen penetrations.

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Mode	Binding Affinity (kcal/mol)	Distance from best mode	Distance from best mode
		Rmsd 1.b.	Rmsd u.b.
1	-1.5	0.000	0.000
2	-1.5	2.812	2.812
3	-1.4	4.368	4.368
4	-1.4	6.891	6.891
5	-1.3	4.379	4.379
6	-1.3	7.559	7.559
7	-1.3	19.359	19.359
8	-1.3	9.964	9.264
9	-1.3	9.325	9.325

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