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Macromolecular Docking Simulation to Identify Binding Site of FGB1 for Antifungal Compounds

Prabhakaran Soundararajan,[†,a] Sugunadevi Sakkiah,[‡,a] Iyyakkannu Sivanesan,[§] Keun Woo Lee,[‡,*] and Byoung Ryong Jeong[†,§,#,*]

[†]*Department of Horticulture, Division of Applied Life Science (BK21 Program), Graduate School, Gyeongsang National University, Jinju 660-701, Korea. [*]E-mail: brjeong@gmail.com*
[‡]*Division of Applied Life Science (BK21 Program), Systems and Synthetic Agrobiotech (SSAC), Plant Molecular Biology and Biotechnology Research Center (PMBBRC), Researh Institute of Natural Science (RINS), Gyeongsang National University (GNU), Jinju 660-701, Korea*
[§]*Institute of Agricultural & Life Science, Gyeongsang National University, Jinju 660-701, Korea*
[#]*Research Institute of Life Science, Gyeongsang National University, Jinju 660-701, Korea. [*]E-mail: kwlee@gnu.ac.kr*
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Fusarium oxysporum, an important pathogen that mainly causes vascular or fusarium wilt disease which leads to economic loss. Disruption of gene encoding a heterotrimeric G-protein-β-subunit (FGB1), led to decreased intracellular cAMP levels, reduced pathogenicity, colony morphology, and germination. The plant defense protein, *Nicotiana alata* defensin (NaD1) displays potent antifungal activity against a variety of agronomically important filamentous fungi. In this paper, we performed a molecular modeling and docking studies to find vital amino acids which can interact with various antifungal compounds using Discovery Studio v2.5 and GRAMM-X, respectively. The docking results from FGB1-NaD1 and FGB1-antifungal complexes, revealed the vital amino acids such as His64, Trp65, Ser194, Leu195, Gln237, Phe238, Val324 and Asn326, and suggested that the anidulafungin is a the good antifungal compound.The predicted interaction can greatly assist in understanding structural insights for studying the pathogen and host-component interactions.

Key Words : NaD1, FGB1, Homology modeling, Molecular docking, Antifungal compounds

Introduction

Fusarium oxysporum, a fungal pathogen that affects a variety of food and ornamental plants. The species consists of non-pathogenic and pathogenic isolates, both known as efficient colonizers of the root rhizosphere. *F. oxysporum* survives in the soil either as dormant propagules (chlamydospores) or by growing saprophytically on organic matter in the absence of plant roots.[1] Fusarium wilt of bananas,caused by *F. oxysporum* f.sp. *cubense* (E.F. Smith) Snyder and Hansen, is a major biotic constraint for banana production.[2] In tomato, fusarium crown and root rot is caused by *F. oxysporum* f.sp. *radicis-lycopersici*.[3] *F. oxysporum* f.sp. *niveum* (E.F. Smith) Snyder and Hansen, cause wilt disease in watermelon.[4] Cotton is one of the major commercial crops across the globe. Wilt of cotton (*Gossypium* spp.) is caused by *F. oxysporum* f.sp. *vasinfectum* (E.F. Smith) Snyder and Hansen. This disease is widespread and causes substantial crop losses in most of the major cotton-producing areas of the world.[5] It also affects other plants such as cabbage (*Brassica* spp.) (*F. oxysporum* f.sp. *conglutinans*), flax (*Linum* spp.) (*F. oxysporum* f.sp. *lini*), muskmelon (*Cucumis* spp.) (*F. oxysporum* f.sp. *melonis*), onion (*Allium* sp.) (*F. oxysporum* f.sp. *cepae*), pea (*Pisum* spp.), (*F. oxysporum* f.sp. *niveum*), China aster (*Calistephus* spp.) (*F. oxysporum* f.sp. *callistephi*), carnation (*Dianthus* spp.) (*F. oxysporum* f.sp. *dianthi*), chrysanthemum (*Chrysanthemum* spp.) (*F. oxysporum* f.sp. *chrysanthemi*), gladioli (*Gladiolus* spp.) (*F. oxysporum* f.sp. *gladioli*) and tulip (*Tulipa* spp.) (*F. oxysporum* f.sp. *tulipae*).[6,7]

Due to the clogging of xylem vessels by *Fov's* pathogenic proteins, water-flow is obstructed and results plant(s) become wilted. In living organisms, G proteins i.e., Guanine nucleotide binding proteins, regulates many vital biological function *via* signal transduction. The G proteins are composed of three sub-units such as α, β and γ. With the help of effectors molecules, the above mentioned G protein sub-units are involved in transmembrane receptor activation. This mechanism controls the gene expression, cellular function and metabolism.[8] In fungi, G protein signaling has been implicated in mediating various biological processes, including cell growth, differentiation and virulence.[9] The G protein's, α subunit (FGA1) and β subunit (FGB1) have partially overlapping functions in the regulation of development and pathogenicity in *F. oxysporum*. Disruption of FGB1 leads to decreased intracellular cAMP levels, reduced pathogenicity and alterations in physiological characteristics, including heat resistance, colony morphology, conidia formation and germination frequency. For managing this lethal disease, few options are already exist. The work conducted in South Africa results, methyl bromide significantly reduced disease incidence, but was effective for only 3 years

[a]Contributed equally as first author

due to recolonization of the fumigated areas by the pathogen.[10] Plant injected by carbendazim and potassium phosphonate have been reported to provide some control, but results have been erratic or unrepeatable. Heat treatment of soil was used recently to control the spread of the pathogen in the Philippines, but this method will likely suffer the fate described for methyl bromide-treated soil.

In practice, diagnosis of plants infected by *F. oxysporum* is carried out by combining diagnostic symptoms seen on the host with the presence of the fungus in the often discolored tissues. The classical approach to overcome this disease is becoming increasingly problematic because more than one *forma speciales* may occur on a given host, along with non-pathogenic strains which are common soil and rhizosphere inhabitants.[11] Besides its well studied activity as a plant pathogen, *F. oxysporum* is known as a serious emerging pathogen of humans due to the increasing number of severe cases reported and to its broad resistance to the available antifungal drugs.[12,13] Now *Fusarium* is represent as the second most frequent mold causing invasive fungal infections in immunocompromised patients, frequently with lethal outcomes.[14-16] *F. oxysporum*, together with *F. solani* and *F. verticillioides*, are responsible for practically all of the cases of invasive fusariosis in humans.[17] Given the dual ability to cause disease both on plants and on humans, we reasoned that *F. oxysporum* could serve as a universal model for studying fungal virulence mechanisms. As per the early report, protein-protein interactions and protein-ligand interactions showed that FGB1 interact more with the aquaporin, a host transmembrane protein which is affected by the *Fov*, then other pathogenic proteins such as FGA1 and FRP1, (F-box protein required for pathogenicity).[18] Because of the presence of NaD1 (*Nicotiana alata* Defensin), a natural antifungal protein, tobacco shows resistant against *F. oxysporum*.[19] Apart from using the resistant proteins; we used human antifungal agents and studied its interaction with FGB1. The antifungal compounds used are amphotericin B (AMB), fluconazole (FLU), itraconazole (ITR), posaconazole (POS), voriconazole (VOR), flucytosine (5FC), caspofungin (CAS), micafungin (MFG) and anidulafungin (ANI). With some exceptions the above mentioned compounds show activity against fungus such as *Aspergillus fumigates*, *A. flavus*, *A. terreus*, *A. niger*, *A. nidulans*, *Candida albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, *C. lusitaniae*, *Cryptococcus* spp., Blastomycoses, Histoplasmosis, Coccidioidomycosis, *Fusarium* spp., Phaeohyphomycoses, *Pichia* spp., *Saccharomyces* spp., *Scedosporium apiospermum*, *Scedosporium prolificans*, *Trichosporon* spp. and Zygomycetes.[20] The AMB binds to the membrane sterols of fungal cells, causing impairment of their barrier function and loss of cell constituents by creating the pores. Metabolic disruption and cell death are consequent upon membrane alterations.[21] Azoles drugs are fungistatic, it limits fungal growth but depending on epidermal turnover to shed the living fungus from the skin surface.[22] The 5FC is deaminated to 5-fluorouracil by fungal cytosine deaminase and the 5-fluorouracil is further converted to 5-fluorodeoxyuridylic acid, which interferes with DNA synthesis of the fungus.[23] The three antifungal compounds (CAS, MFG and ANI) of above mentioned are come under the class Echinocandins. The echinocandins are synthetically modified lipoprotein, originally derived from fermentation broths of various fungi.[24] These are inhibitors of beta-(1, 3)-glucan synthesis, an action that damages fungal cell walls.[25]

Computational techniques are one of the vital processes to understand interaction between the protein(s) and drug(s).[26] *In silico* screening of drugs help a lot for reducing the number of candidates molecules for synthesis and experimental result. Molecular docking is the optimal process in way of fast alternatives and also inexpensive method to design a novel scaffolds.[27] In this paper we reported the best antifungal compounds for *F. oxysporum* based on structural interactions such as protein-protein and protein-ligand studies. The Discovery Studio v2.5 (DS) was used for molecular modeling of FGB1 and to study the protein-ligand interactions.[28] Because of the unavailability of protein structural details, initially protein-protein interaction study was carried to find the active site or binding site of FGB1 using Q-SiteFinder and GRAMM-X.[29,30] LigandFit module from DS was used to dock the various antifungal compounds in the active site of FGB1[31] and selected the best compounds based on the multiple scorings functions and binding affinity, which is explained in upcoming parts. Consensus scoring method is one of the best ways to select the suitable pose of the small molecules in the active site of the target protein. For improving the probability of identifying 'true' ligands and to balance errors in single scores, consensus scoring combines information from different scores.[32] This study is fully focused on the refinement of interactions between FGB1 and antifungal (ligand/receptor) compounds and find the vital amino acid *via* molecular docking.

Materials and Methods

Homology Modeling. Due to the absence of X-ray crystal structure of FGB1 in Protein Data Bank (PDB, www.rcsb.org), homology model was carried out to find its tertiary structure.[33] Tertiary structure describes the folding and assembly of the different secondary structure elements like α-helices, β-sheets and loops. In multi-domain proteins, tertiary structure includes the arrangement of domains relative to each other as well as that of the chain within each domain. The FGB1 protein primary sequence was retrieved from Swiss-Prot Protein Database (Accession ID: Q96VA6) which has 359 amino acids. The identity and similarity between the target and the template protein determines the quality of the homology model structure.[34] To find a suitable template for FGB1 a similarity search against PDB was performed using BLAST server; the selected template was used to build a three dimensional structure of FGB1 using Discovery Studio v2.5. CHARMm force field was used to estimate the energy and stability of modeled structures[35] and the resultant model was checked using the Ramachandran

plot.[36]

Active Site Prediction. The region of a protein that interacts with a ligand is generally referred to as the "active site". Active site prediction is one of the vital processes in drug designing methodologies such as molecular modeling studies, pharmacophore modeling, and comparison of functional sites. Generally, active site lies on the surface and/or buried inside the protein. For finding the interaction site in the FGB1, we used Q-SiteFinder, an online web server. It uses the interaction energy between the protein and a simple van der Waals probe to locate energetically favorable binding sites. Energetically favorable probe sites are clustered according to their spatial proximity and then the clusters are ranked according to the sum of interaction energies for sites within each cluster.[29]

Molecular Docking Simulation. In this work two types of molecular docking were used such as macromolecular (protein-protein) docking and protein-ligand docking. GRAMM-X, web server was used to dock the FGB1 and NaD1 for confirming the active site predicted from the Q-SiteFinder.[30] *LigandFit* from DS was used to dock the nine anti-fungal compounds in the binding site of FGB1.[31]

Protein-Protein Docking Simulation. GRAMM-X, is used to predict the protein-protein interaction site in protein surface, requires only atomic coordinates of two molecules. The molecular pairs may be: two proteins, a protein and a smaller compounds, two transmembrane helices, etc. GRAMM-X uses an empirical approach to smoothing the intermolecular energy function by changing the range of the atom-atom potentials. The technique locates the area of the global minimum of intermolecular energy for structures of different accuracy. The quality of the prediction depends on the accuracy of structures. GRAMM-X was able to detect the near native matches in complexes with large conformational changes. Maximum numbers of 100 models were saved using the default parameters.

Protein-Ligand Docking. Docking was carried out to find the suitable orientation and interactions (hydrogen bond and hydrophobic interaction) of the lead in protein active site. LigandFit, one of the best docking techniques was used to dock the screened compounds.

Nine antifungal compounds were selected from the literatures to dock into the binding site of FGB1 protein. The 2D structure of all small molecules (ligands) were built using MDL-ISIS Draw v2.5 and imported into DS for the 3D format conversion. Maximum of 255 conformations were generated for each compound using the Best Conformation model generation method based on CHARMm force field's Poling algorithm.[37] To ensure energy-minimized conformer of the molecules, the conformation with energy more than 20.0 kcal/mol from the global minimum was rejected and molecules with their lowest energy conformations were selected for the docking studies.

The quality of receptor structure plays a central role in determining the success of docking calculations.[38,39] In general, higher the resolution of the employed crystal structure, better the observed docking results. There are three stages in *LigandFit* protocol: (i) Docking: Attempt. is made to dock a ligand into a user defined binding site, (ii) In-Situ Ligand Minimization and (iii) Scoring: Various scoring functions were calculated for each pose of the ligands. Initially receptor was prepared by applying CHARMm force field using DS.[40] After protein preparation, binding site of the protein has to be identified to dock the small molecules. The active site of protein can be represented as binding site; it is a set of points on a grid that lie in a cavity. Two methods are usually applied to define a binding site for a protein: (i) Based on the shape of receptor using "eraser" algorithm and (ii) Based on volume occupied by the known ligand pose already in an active site.[41,42] Here the first method was used to define the binding pocket of FGB1. We set the maximum poses retained as 10, RMS threshold for diversity as 1.5 Å and score threshold for diversity 20.0 kcal/mol. The minimization algorithm has been set and the minimization energy tolerance was assigned to zero and 0.001 for the gradient tolerance. The primary goal of scoring is to outline the border of binders against non-binders. The prediction of the ranking of ligands corresponding to their activity is of (grammer) lower interest, since it is the main importance in result interpretation, scoring functionswere implemented in the *LigandFit* module. In our study, various scoring has been used as an indicator to select the best antifungal compounds like LigScore1 and LigScore2, Piecewise Liner Potential 1 (PLP1), Piecewise Linear Potential 2 (PLP2), Jain, Potential of Mean Force (PMF), Ludi and Dock scores.[43-48] Based on the scoring functions and the appropriate interactions with the critical residues were considered as a final cut off requirements to select the best antagonist for FGB1.

Result and Discussion

Homology Modeling. As mentioned earlier, FGB1 is an essential pathogenesis protein in *F. oxysporum*. It plays a vital role in gene expression, cellular function and metabolism such as cAMP level, heat resistance, colony morphology and conidia formation, and germination frequency. Still now there is no 3D structure of FGB1 protein was deposited in PDB, hence homology model was performed to determine the 3D coordination of FGB1. Using BLASTp search 2BCJ (PDB ID), *Bos taurus* G-Protein-Coupled receptor 2 was selected as a best template, which shown an identity of 67% as well as it has similar functions and the total number of amino acids are 339. ClustalW was used for the sequence alignment between the template and the target protein (Fig. 1). The aligned sequence was a input in Built Homology Model/DS and the 3D structure of FGB1 was generated. As mentioned earlier, FGB1 is the subunit of G protein, an essential protein for cell signaling and other vital processes.[8] Both FGA1 and FGB1 units have the potent to alter the activity of a diverse set of effector molecules such as adenylate cyclase, ion channels and phospholipases.[49] The modeled protein has helices on 1-25aa and 30-37aa, which are connected by loop. After the single beta strand located in region between 48-53aa, a barrel like structure was formed

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Target    MNSQGNSDVSPEAMQSRIQQARREAETLKDRIKRKKDDLADTTLRAVAQQAHEPIPKNQL 60
Template  ---------------SELDQLRQEAEQLKNQIRDARKACADATLSQITNN-IDPVGR-IQ 43
                          *.::* *:*** **::*:  :.  **:**  :::: :*: :

Target    MKAKRTLKGHLAKIYAMHWSTDRRHLVSASQDGKLIIWDAYTTNKVHAIPLRSSWVMTCA 120
Template  MRTRRTLRGHLAKIYAMHWGTDSRLLVSASQDGKLIIWDSYTTNKVHAIPLRSSWVMTCA 103
          *:::***:***********.** * ***************:********************

Target    YAPSGNFVACGGLDNICSIYNLNQQRDGPTRVARELSGHAGYLSCCRFINDRSILTSSGD 180
Template  YAPSGNYVACGGLDNICSIYNLKT-REGNVRVSRELAGHTGYLSCCRFLDDNQIVTSSGD 162
          ******:***************:  *:* .**:***:**:*********:::*..*:*****

Target    MTCMKWDIETGQKVTEFADHLGDVMSISLNPTNQNTFISGACDAFAKLWDIRAGKAVQTF 240
Template  TTCALWDIETGQQTTTFTGHTGDVMSLSLAPDTR-LFVSGACDASAKLWDVREGMCRQTF 221
            **  *******:.* *:.* *****:** * .:  *:****** *****:* * . ***

Target    AGHESDINAIQFFPDGHSFVTGSDDATCRLFDIRADRELNLYGSESILCGITSVATSVSG 300
Template  TGHESDINAICFFPNGNAFATGSDDATCRLFDLRADQELMTYSHDNIICGITSVSFSKSG 281
          :********* ***:*::*.************:***:**  *. :.*:******: * **

Target    RLLFAGYDDFECKVWDITRGEKVGSLVGHENRVSCLGVSNDGTSLCTGSWDSLLKIWAY 359
Template  RLLLAGYDDFNCNVWDALKADRAGVLAGHDNRVSCLGVTDDGMAVATGSWDSFLKIWN- 339
          ***:*******:*:***  :.::.* *.**:********::** ::.******:****
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Figure 1. Alignment of FGB1 (*F. oxysporum*) sequence with homologues 2BCJ (*Bos taurus*) shared 67% of identity and has same function with the protein FGB1. Conserved residues are represented by asterisk (*), gaps are represented by hyphen (–), strong and weak conserved amino acids are denoted by (:) and (.), respectively.

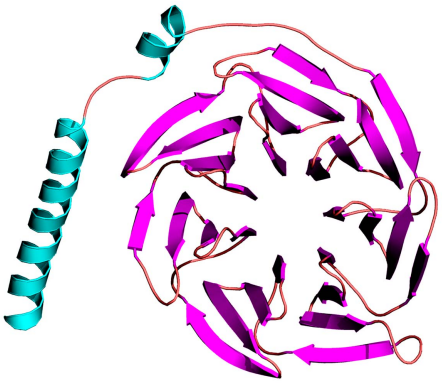
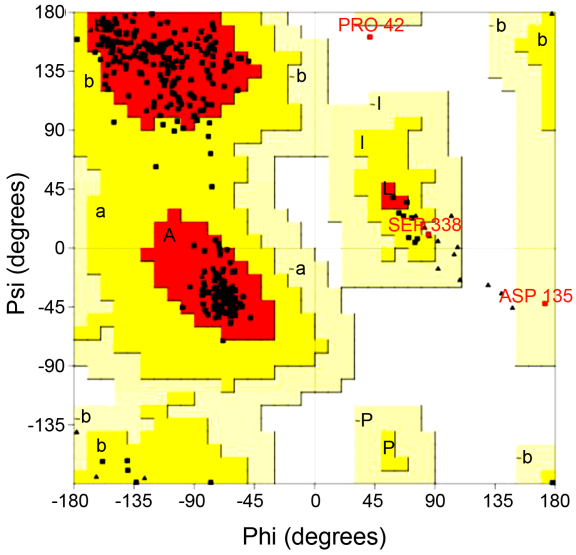


Figure 2. The three dimensional structure of protein FGB1 (*F. oxysporum*), modeled using DS and G Protein-Coupled Receptor Kinase 2 (PDB ID: 2BCJ) was used as the template.



Residues in most favoured regions [A,B,L]	281	90.9%
Residues in additional allowed regions [a,b,l,p]	26	8.4%
Residues in generously allowed regions [-a,-b,-l,-p]	2	0.6%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	309	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	26	
Number of proline residues	7	
Total number of residues	344	

Figure 3. Ramachandran plot to validate the theoretical model of FGB1 protein. It showed, 90.9% of amino acids are in favored regions, 8.4% are in additional allowed regions, 0.6% in generously allowed regions and no amino acids are presented in disallowed regions. Note: In the good model more than 90% of amino acids are in allowed regions.

by seven consecutive sheets, each consist of four anti-parallel beta strands and all are connected by loop (Fig. 2). The cartoon representation of FGB1 was depicted in Figure 2. All alpha-helices and beta-sheets, and the backbone structure resembling the same alignment that had been found in the template structure. The final model was validated using the PROCHECK, to find deviations from normal protein conformational parameters.

Validation of Homology Model of FGB1. Ramachandran plot was used to validate the modeled protein structure by checking the detailed stereo chemical quality of a protein structure on residue-by-residue basis.[34] The phi and psi distribution of Ramachandran plot of non-glycine and non-proline residues were summarized in Figure 3. A good homology model should have > –90% of the residues in the favorable region, in our homology model 90.9%, 8.4% and 0.6% of the residues were present in favored, allowed and generously allowed regions, respectively, and relatively only low percentage of residues having general torsion angles which affirms that FGB1 model was accurately predicted. None of the residues were present in the disallowed region and also all the bond distances and angles lie within the allowable range about that standard dictionary values which indicated that FGB1 model was reasonably good in geometry and stereochemistry. The main chain parameters plot for the model was shown in Figure 4 which indicates that the structure compares with well-refined structures at a similar resolution. The six properties plotted: (i) Ramachandran plot quality, at the resolution of 2.0 Å, 90.9% of residues were located in allowed region. Number of band width from mean was 0.7. (ii) Peptide bond planarity, its parameter value and number of band width from mean was 4.0 and –0.5. (iii) Bad non-bonded interactions, this data gives information about the bad contacts per 100 residues. Totally, 1.2 amino acids have bad contacts and its number of band width from mean was –0.3. (iv) Cα tetrahedral distorsion, by using alpha carbon tetrahedral distortion, zeta angle deviation for protein model was measured. Its standard deviation was 1.3 with –0.2 mean band widths. (v) Main chain hydrogen bond energy, its standard deviation was measured in kilo calorie per mol, it's energy and mean value was 0.8 ~ –0.2, respectively and (vi) The overall G factor was in negative. Its parameter value was –0.1 and its number of band widths from mean was 0.9, which measures overall normality of the structure. In brief, the geometric quality of the backbone conformation, the residue interaction, the residue contact

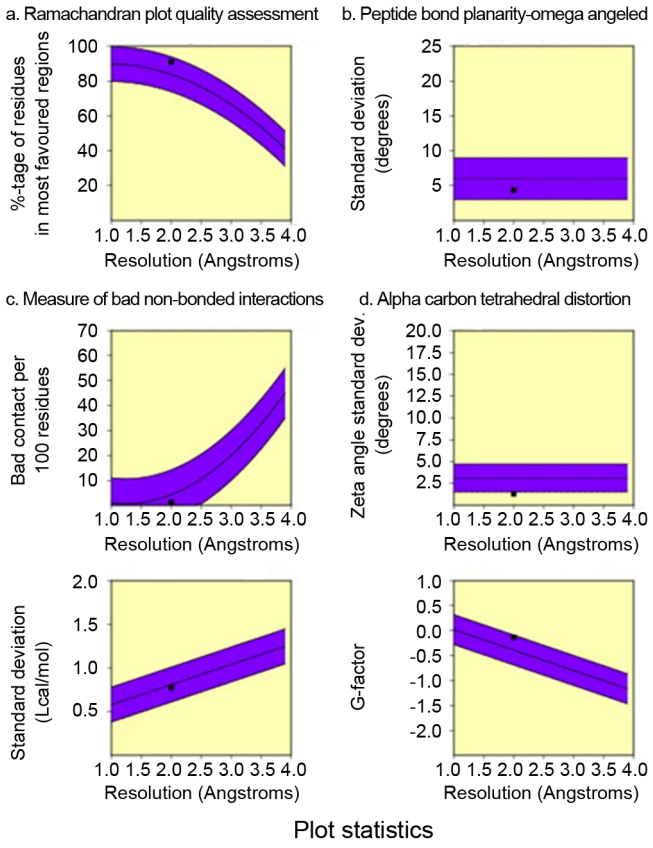


Figure 4. The percentage-target residues in A,B,L are 90.9, Omega angle standard deviation value is 4.4, Bad contacts/100 residues are only 1.2, Zeta angle deviation standard deviation is 1.3, Hydrogen bond energy standard deviation is 0.8 and Overall G-factor is −0.1.

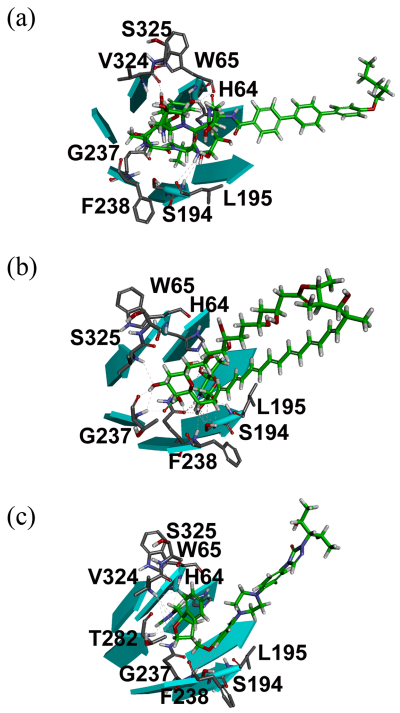


Figure 5. The illustration of the docked pose of antifungal compounds (green-stick) in the active site of FGB1 (only beta-barrel was shown for clarity) and the important amino acids are shown in stick. (a) Amphotericin B (b) Anidulafungin, and (c) Posaconazole.

and the energy profile of structures were well within the limits which established for reliable structures. All the evaluation suggests that a reasonable homology model for FGB1 has been obtained to allow for examination of protein-protein interactions. Root Mean Square Distance (RMSD) between the sequence and the structure was 0.08 Å. The Chi1-Chi2 Plots from PROCHECK was used to check the conformations of each amino acids present in FGB1. Out off 359 amino acids, three amino acids such as Ile (259), Leu (161) and Tyr (107) were in unfavorable conformation which had shown a value less than −3.00. The distorted Geometry of main-chain bond length and main-chain bond angles of C–N, CA–C, CA–C, CA–C and CA–CB was 0.054 Å, 0.053 Å, 0.055 Å, 0.067 Å and 0.055 Å, respectively. By considering the main chain parameters, the values indicated that the generated model was ideal.

Molecular Docking Simulation.

Protein-Protein Docking. Protein-protein docking, the task of predicting 3D structure of protein complex from its component structures which is much useful in the absence of an experimental structure to provide insights into the molecular function of proteins such as the basis for recognition and affinity. In GRAMMX, FGB1 was assigned as a receptor and the NMR structure of NaD1 was selected as a ligand protein. An important feature of GRAMM-X is the ability to smooth the protein surface representation to account for possible conformational change upon binding within the rigid body docking approach. This program performs an exhaustive 6-dimensional search through the relative translations and the rotations of the molecules.[30] The NaD1 has 20 monomers and all were docked with FGB1 receptor and 100 docked poses were collected for each monomer. Out of 2000 docked complex, nearly 700 poses of NaD1 were docked at the top of the FGB1. Hence one representative complex from 700 poses was selected based on its energy values and used as a reference complex structure (Model 1) for further protein-ligand docking studies (Fig. 5).

Protein-Ligand Docking. Molecular docking is one of the best post filtration methods in drug design process. The protein-ligand docking study was performed to check how well the small synthetic compounds can be an effective antagonist as NaD1 docked with FGB1. Due to the inaccessibility of literature information about the active site in FGB1 protein, protein-protein docking was carried out and the best complex was selected as a receptor for ligand docking. The natural antifungal protein, NaD1 showed interactions with His64, Trp65, Thr67, Asp68, Ser194, Leu195, Asn196, Pro197, Gln237, Phe238, Phe239, Pro240, Asn241, Gly242, Val284, Val324, Ser325, Asn326 and Asn327 of FGB1. The interacted site in FGB1 with the NaD1 might be concluded as an important region which is involved/response for antagonist/agonist activities. This putative binding site was used against the nine antifungal compounds such as AMP, ANI, CAS, FLU, ITR, MFG, POS, VOR and 5FC. These anti-

Table 1. Amino acids of FGB1 interacts with the given protein and ligands

Protein/Ligand name	His64	Trp65	Ser194	Leu195	Gln237	Phe238	Val324	Asn326
NAD1	Y	Y	Y	Y	Y	Y	Y	Y
Amphotericin B	Y	-	Y	Y	Y	Y	Y	Y
Flucytosine	Y	Y	Y	Y	Y	Y	Y	Y
Posaconazole	-	Y	Y	Y	Y	Y	Y	Y
Voriconazole	Y	-	Y	Y	Y	-	Y	Y
Fluconazole	-	Y	Y	Y	Y	Y	-	-
Itraconazole	-	Y	-	-	-	Y	-	Y
Micafungin	No Interactions							
Anidulafungin	Y	Y	Y	Y	Y	Y	Y	Y
Caspofungin	No Interactions							

Table 2. Consensus scoring values for best ligands

Compound name	Ligscore1	Ligscore2	PLP1[a]	PLP2	Jain	PMF[b]	Dock score	Ludi
Amphotercin B	5.32	6.35	112.42	105	1.54	49.08	112.99	567
Posaconazole	3.15	6.14	116.46	94.37	1.3	24.91	109.65	468
Anidulafungin	8.46	7.7	145.68	132.21	4.27	103.63	132	628

[a]PLP-Piecewise Linear Potential. [b]PMF-Potential of Mean Force.

fungal compounds were considered as small molecules and used to find the active amino acids present in FGB1's putative binding site. Here we noted the common amino acids in the FGB1 interact with both NaD1 and antifungal compounds (Table 1) may be considered as the critical amino acids required for causing pathogenesis by *F. oxysporum* on the host organisms. Visual inspection of the molecular docking solutions has been carried out and two compounds such as MFG and CAS were eliminated because of not able to produce the interactions with FGB1. LigScore1 and LigScore2 were calculated by the descriptors of polar surface in receptor-ligand interactions.[43] Piecewise Linear Potential (PLP) score has been calculated by the descriptions about hydrogen bonds forming. Higher PLP score indicated stronger receptor-ligand binding.[44] Potential of Mean Force (PMF) score was calculated by the summing pair wise interaction terms over all inter-atomic pairs of the receptor-ligand complex.[45] Jain and Ludi scores were consulted in hydrophobic interaction and degree of freedom, respectively.[46,47] Docking score was considered as the degree of difficulty about ligand moving into the binding site.[48] The ANI owns the highest docking score such as 132 while AMB and FLU score was 112.99 and 109.65, respectively. Not only for docking score, ANI placed top also in Ligscore1, Ligscore2, PLP score, Jain, PMF, and Ludi (Table 2). There is no significance difference between the Ligscore1 and Ligscore2. Usually molecular docking relies strongly on the robust and accurate scoring function, that can be used to identify the correct binding mode and prioritize the docked molecules.[50] For choosing the best interacting compound, we correlates the amino acids involved in the interaction between NaD1-FGB1 complex, FGB1-antifungal complex (Fig. 5), and the binding affinity by means of different scorings. Finally, anidulafungin (ANI) was concluded as a best antifungal compound, because of highest binding affinity and also bind with most of the amino acids which were interacted in FGB1-NaD1 complex. As the binding affinity is high, the activity of ANI also might be higher than other compounds or much close to NaD1. By estimating all docking results, amino acids such as His64, Trp65, Ser194, Leu195, Gln237, Phe238, Val324, and Asn326 of FGB1 were considered as important and directly involved in interactions with antagonist (Fig. 5). So, that the drug discovery aspects will be more easier, if we target the above mentioned amino acids. Yet the molecular biology study should be done to prove the exact activity and mechanism of this protein and ligand interaction.

Conclusions

Fusarium wilt has been a major limiting factor in the production of agricultural and horticultural crops.[6,7] The FGB1 is the vital protein for causing pathogenicity. While *Nicotiana alata*, contains the natural defensin NaD1, an antifungal protein against filamentous fungi including *F. oxysporum* f.sp. *vasinfectum*.[19] Nearly, 20 different conformers of NaD1 were docked with FGB1 and the binding site was selected by comparing active site predicted from Q-SiteFinder with the maximum number of the NaD1 conformation binding. From the result of Q-SiteFinder and protein-protein interaction between NaD1 and FGB1, we found the binding site and the amino acids which were interacted with FGB1. The selected binding site was used to dock the nine antifungal compounds. By analyzing the amino acids involved in both protein-protein and protein-ligand docking, His64, Trp65, Ser194, Leu195, Gln237, Phe238, Val324, and Asn326 of FGB1 were considered as critical amino acids required for pathogenetic effect. Out of nine compounds, amphotericin, posaconazole and anidulafungin, form hydrogen bonds with most of the critical

residues of FGB1. In these three compounds, ANI interacts more efficiently than others. This ligand may act as best antifungal agents for *F. oxysporum*. The mechanism of how the antifungal compounds interact with the FGB1 is revealed here. Nonetheless these data require confirmation by appropriate experimental data. Our theoretical prediction may lead to the establishment of molecular biology approaches.

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