Docking Studies on the C-AMP – Dependent Protein Kinase A (CPKA) Of Fungus MagnaportheOryzae the Causative Agent of Rice Blast Disease in Oryza Sativa.

Tapas Ranjan Samal[1], M Jagannath[*2], K Umadevi[3]

Abstract

The fungus Magnaportheoryzae (Phylum: Ascomycota; order: Magnoporthales; family: Magnaporthaceae) is the causative agent of rice blast disease in Oryza sativa. The infection is initiated by a structure called appressorium produced by the mycelia of M. oryzae in its incubation periods. The earlier studies indicates that the enzymes Adenylate cyclase (MAC1) and catalytic sub unit of CAMP dependent protein kinase A (CPKA) were very essential for development of appressorium and parthenogenesis. So these proteins can be considered as target proteins for the development of drug to control the rice blast disease. In this study, docking analysis is carried out to investigate the atomic interaction and molecular mechanism between seven chemical compounds namely Balanol, Genistein, H8, H88, H89, Isoquinoline and Staurosporine and CPKA protein of Magnaportheoryzae. It was undertaken in three different components. a. Modeling of CPKA protein structure using phyre 2 server, Energy minimization and protein structure refinement by ModRefiner algorithm i.e., finding the molecular interaction between CPKA protein of M. oryzae and different ligands of the above said seven chemical compounds, using AutoDock 4.2 tool.

KeyWords: Ankylosing Spondylitis, studies, docking,

DOI Number: 10.14704/nq.2022.20.11.NQ66006 **NeuroQuantology2022; 20(11):50-54**

Background

The fungus Magnaportheoryzae (Phylum: Ascomycota; order: Magnoporthales; family: Magnaporthaceae) is the causative agent of rice blast disease in Oryza sativa. The infection is initiated by a structure called appressorium produced by the mycelia of M. oryzae in its incubation periods. Appersorium is the structure that penetrates into the host plant cells and initiate infection. After entering into the host cells, this structure releases numerous effectors into the host plant tissues, which suppresses the plant immunity (1).

When conidia of the above mentioned species of fungus is attached to a rice blade, a germ tube arise initially from the conidia. The polar elongation of germ tube will be stopped subsequently, the tip of the germ tube will be swollen to form an appressorium, surrounded by a thick melanized cell wall (2). Turgor pressure will be developed inside the appressorium, passing the swollen bulb like tip into the plant tissue. This is followed by an invasive growth of the mycelia of fungus inside the plant tissue (3, 4).The mitogen activated protein kinase (MAPK) and the c-AMP - dependent signaling pathways (5,6) governs the pathogenicity and appressorium development of M. oryzae.

Corresponding author: M Jagannath
Address: [1]Trainee, DBT-BIF Programme, Department of Marine Living Resources, Andhra University, Visakhapatnam, 530003, India,[2]Research Assistant(SRF), DBT-BIF Programme, Department of Marine Living Resources, Andhra University, Visakhapatnam, 530003, India.[3]Associate Professor and Coordinator DBT-BIF Programme, Department of Marine Living Resources, Andhra University, Visakhapatnam, 530003, India
E-mail: m.jagannath1@gmail.com

The enzyme PMK1 involve in the formation of appressorium and MPS1 is required at the time of penetration into the host plant tissue (7,8).The mitogen activated protein kinases such as PMK1 of M. oryzae is a homolog of FUS3/KSS1 proteins of Saccharomyces cerevisiae and the MPS1 of M. oryzae is the homolog of SLT2 of S. cerevisiae (7,8).

The enzyme Adenylate Cyclase (MAC1) is responsible for release of ATP from cAMP which activates the cAMP – dependent protein kinase A (CPKA), which is essential for appressorium formation in M. oryzae. The enzyme CPKA in M. oryzae exists as an inactive tetramer consists of two monomers as catalytic subunits and two monomers as regulatory subunits. The catalytic submit of CPKA is released upon binding of cAMP to the regulatory subunits (9) and thus activates the formation of the appressorium in M. oryzae.

The CPKA gene is of 1894 bp length and contains three short introns at nucleotides 963 to 1044, 1299 to 1410 and 1786 to 1866. This structure of CPKA allows a potential protein of 539 amino acids with a predicted molecular mass of 60.7 kD to be encoded (10). The catalytic core region was identified between the amino acids 228 and 483. 81% of this catalytic core region is homologous to the TPK1, TPK2 and TPK3 proteins of S. cerevisiae. The N- terminal region of CPKA contains large number of glutamine residues. The glutamines in CPKA were present as strings alternating Proline or Serine residues.

In CPKA, the ATP binding motif starts at position 236 Gly – Thr – Gly – Ser – Phe – Gly – Arg – Val – (16 residues) – Lys; a catalytic loop motif starts at position 350 Arg – Asp – Leu – Lys – Pro – Glu – Asn; a magnesium ion chelating loop starts at position 368 Asp – phe – Gly – Phe and residues such as Glutamine at position 263, Asparginine at 405 position & Arginine at 466 position, which are important in protein folding (12).

The earlier studies indicates that the enzymes Adenylate cyclase (MAC1) and catalytic sub unit of cAMP dependent protein kinase A (CPKA) were very essential for development of appressorium and parthenogenesis. So these proteins can be considered as target proteins for the development of drug to control the rice blast disease. A variety of chemical compounds have been used as fungicides to control rice blast disease in Oryza sativa, but their exact made of functioning on the mycelia of M. oryzae was not fully understood. It is essential to explore the interaction profile of those chemicals with the proteins of M. oryzae for proposing novel drugs of chemical derivation that could be effective in preventing or controlling the blast disease without harmful effects to the animals standing in the higher trophic levels of the food chain.

Variety of fungicide chemicals have been used to reduce the infection caused by Magnaporthe, inhibiting the function of catalytic subunit of CPKA protein. Among all those, chemical compounds such as balanol, genistein, H8, H88, H89, isoquinoline, staurosporine have been reported to impair the functioning of cAMP – dependent protein Kinase A (CPKA) of M. oryzae.

In this study, docking analysis is carried out to investigate the atomic interaction and molecular mechanism between seven chemical compounds namely Balanol, Genistein, H8, H88, H89, Isoquinoline and Staurosporine and CPKA protein of Magnaportheoryzae.

This study was undertaken in three different components. a. Modeling of CPKA protein structure using phyre 2 server, b. Energy minimization and protein structure refinement by ModRefiner algorithm i.e., finding the molecular interaction between CPKA protein of M. oryzae and different ligands of the above said seven chemical compounds, using AutoDock 4.2 tool.

Methods

CAMP – dependent protein kinase A (CPKA)

CPKA protein of Magnaportheoryzae was chosen as drug target. The sequence information of the protein of M. oryzae CPKA (Identifier: Q01143) was extacted and downloaded from UniPort KB (https://www.uniport.org/help/uniportkb).

Molecular modeling and structure validation of target protein

Tertiary structure of CPKA protein of M. oryzae was predicted by using Phyre2 Server, Modeler 9.17 and Swiss model. These structures were further validated by proSA – web [13], proQ [14], PROcheck [15] and ERRAT2[16]. For energy minimization and protein structure refinement of the predicted model, ModRefiner algorithm was adapted.

Owing to the unavailability of experimentally determined structure of selected 539 amino acid long CPKA protein of M. oryzae, the 3^0 structure

was forecasted using the Phyre2 online tool following standard protocol, followed by refinement of the structure and minimization of energy was undertaken subjecting the predicted structure to the ModRefiner algorithm.
The Z score value of CPKA protein was evaluated using proSA – Web evaluation. The 3D model of M. oryzae CPKA protein was obtained by proQ tool.

Preparing the Ligand for molecular docking

The seven chemical compounds (Table.....) reported to act against M. oryzae CPKA protein were searched and downloaded from the PubChem database (https://pubchem. ncbi.nim.nih.gov/) and CASc Ds (Chemical Abstract Service (ds)). The 2-D structures were prepared with the help of ACD/chemsketch (http://www.acdlabs.com/). The 2-D structures thus obtained were converted to 3-D structures by using OpenBabel Program (19). The generated structures were then subjected to energy minimization by UCSF chimera tool. Table 2 shows the chemical ligands used against Magnoportheoryzae.

Binding Site Analysis

Identification of protein – ligand binding site is the principal task in the drug designing and docking algorithm. In the past two decades, many approaches have been developed to identify the protein – ligand binding site in the process of drug designing. In this study, CASTP (http://cast.engr.uic.edu) web server was used to predict the protein –ligand binding site of CPKA protein of M. oryzae (20). These binding sites were also referred as activesites. For further virtual screening and docking studies, selection was made out of these activesite residues.
Both ligands and receptor molecules were prepared for docking analysis using AutoDock 4.2 program (22). Docking studies were performed adapting standard methodology for protein – ligand docking (23). The docking analysis results for the structures was confirmed basing on the binding energy. The lowest binding energy conformation was selected. ADT tool and LigPlot were employed for the visualization of structure files and to prepare schematic diagram of protein ligand interactions (24).

Results and Discussion

CAMP – dependent protein kinase A (CPKA)

The protein sequence of M. oryzae protein CPKA with 1d Q01143 was retrieved from uniprotKB is having an amino acid sequence length 539 and 60.756 D of unprocessed protein mass. The relative arrangement of atoms of the refined model of CPKA using ProCheck analysis (Figure 1c) shows that 91.5% residues were located in the most allowed or favorable region, 6.9% residues in additional allowed regions, 0.4% residues were in the generously allowed region and 1.2% residues fell in the disallowed region of the Ramachandran Plot (Fig. 1c). ProSA – web evaluation revealed a compatiable Z score value of -8.07 (Fig. 1b) which is within the range of native conformation of crystal structures.

Molecular modeling and structure validation

The 3D model of CPKA protein of M. oryzae showed a LG (Lovitt – Gerstein) score 5.448 and MAXUS 0.539 as given by Protein Quality Predictor (proQ. tool) which implies a high accuracy of the predicted structure. To rate a model as extremely good model, proQ LG score >4 is necessary (18). An overall quality for CPKA protein was found to be 61.232 as achieved using the ERRAT2 plot (Fig.1d). All the above results recommended that the predicted model of CPKA is reliable. The query coverage and validation results obtained revealed that Phyre2 server gave a reliable result when compared to the Modeler 9.17 and Swiss model.

Ligand preparation and molecular docking

The results of the current study revealed that the structure demonstrates different conformations with different binding energy. The least binding energy conformation was chosen. Docking studies on ligands of the 7 different chemical compounds revealed that Staurosporine showed the lowest binding energy (i.e., -10.59 units) with constant of inhibition 17.21 µM for the protein CPKA – ligand complex. The interaction of Staurosporine with 236th amino acid residue Threonine and 308th amino acid Valine forming 2 hydrogen bonds is depicted in the figure 3g.
Staurosporine was first reported to be a microbial alkaloid produced by Streptomyces species. It contains an indol [2, 3-a] carbazole chromophore. The molecular formula of Staurosporine is C28 H_{26}

N_4 O_3 and molecular weight is 466.541g/mol. This compound show low solubility in water and soluble in dimethyl sulphoxide (DMSO) or dimethyl formamide (DMF) [25].

Staurosporine inhibits the catalytic domain of. Staurosporine is the most potent inhibitor against the release of CPKA protein of Magnoportheoryzae which is responsible for the formation of appressorium.

The second compound was H89 (N-[2-(P-bromocynnamylamino) ethyl] -5-isoquinoline sulphonamide) which exhibited low binding energy next to Staurosporine i.e., -10.3 units with an constant for Inhibition of 28.37 µM. The interaction of H89 protein kinase A with amino acid Asp306 and Glu312 is depicted in figure 2c. H89 compound is a derivative of H8 which is a specific inhibitor of cyclic nucleotide dependent protein kinase. H89 is thirty times more potent than H8 in inhibiting the activity of CPKA protein of M. oryzae with ATP (26).

The binding energy of CPKA protein of M. oryzae and liquid complex was examined to be -9.37 units and the constant for inhibition is 135.93 µM. It forms five hydrogen bonds with amino acid residues namely hys257, Thr368, Ser238, Asp369 and Asn 356 of CPKA (Fig. 3a).

The other four chemical compounds like H88, Isoquinolin, H8, Genistein also have a strong inhibiting activity towards M. oryzae CPKA protein with considerable binding energies -8.1, -6.62, -8.06 and -7.3 Kcal/mol respectively (Table 2).

The results of present In Silico studies shows that all the selected compounds inhibited CPKA protein of M. oryzae with considerable binding energies (Table 2). Thus the interaction between the chemical compound and the mycelia of M. oryzae might prevent CPKA activity for appressorium formation, which is the reason for using these chemicals to prevent or control the effect of M. oryzae fungus on rice plants without undesirable effect on human beings.

Binding Site Analysis:

34 active sites were identifies in the target protein CPKA of M.oryzae. These active site-residues of the receptor are 234Leu, 235gly, 236 Thre, 237Gly, 238ser, 239phe, 240gly, 241arg, 242val, 255ala, 257lys, 259leu, 269gln, 272his, 237thr, 276glu, 287ile, 305meth, 306asp, 308val, 311gly, 312glu, 351asp, 371gly, 372ph and 385gly. These active sites were selected for virtual screening and docking studies.

The Computational approach of docking employed in the present study reveals the molecular interaction of these chemicals against CPKA protein. Attention may perhaps be given to CPKA protein in proposeing novel drugs from different chemical resources towards M. oryzae fungal infection. The current docking study suggests that Staurosporine being docked with significant and minimum binding energy (-10.59 Kcal/mol), forming 5 hydrogen bonds with amino acid residues. Threonine236 and valine308 residues of the receptor chemical can be reckoned for both in-vitro and in-vivo authentication.

Acknowledgements

The authors are thankful to the Department of Biotechnology (DBT), Bioinformatics Division, Govt. of India, New Delhi for financial support and also thankful and grateful to Dr. K.Umadevi, Associate professor, Department of Marine Living Resources, and coordinator, DBT-BIF Programme, Andhra University, for final correction of manuscript.

References

Li X, Gao C, Li L, Liu M, Yin Z, Zhang H, et al. (2017) MoEnd3 regulates appressorium formation and virulence through mediating endocytosis in rice blast fungus Magnaportheoryzae. PLoSPathog 13(6): e1006449.

R. D. Kulkarni Æ R. A. Dean Identification of proteins that interact with two regulators of appressorium development, adenylate cyclase and cAMP-dependent protein kinase A, in the rice blast fungus Magnaporthe grisea. Mol Gen Genomics (2004) 270: 497–508.

Howard RJ, Ferrari MA, Roach DH, Money NP (1991) Penetration of hard substrates by a fungus employing enormous turgor pressures. Proc Natl Acad Sci USA 88:11281–11284.

DeJong JC, McCormack BJ, Smirnoff N, Talbot NJ (1997) Glycerol generates turgor in rice blast. Nature 389:244–245

Xu JR, Hamer JE (1996) MAP kinase and cAMP signaling regulate infection structure formation and pathogenic growth in the rice blast fungus Magnaporthe grisea. Genes Dev 10:2696–2706.

Choi W, Dean RA (1997) The adenylate cyclase gene MAC1 of Magnaporthe grisea controls appressorium formation and other aspects of growth and development. Plant Cell 9:1973–1983

Xu JR, Staiger CJ, Hamer JE (1998) Inactivation of the mitogenactivated protein kinase

Mps1 from the rice blast fungus prevents penetration of host cells but allows activation of plant defense responses. Proc Natl Acad Sci USA 95:12713–12718

Thines E, Weber RWS, Talbot NJ (2000) MAP kinase and protein kinase A-dependent mobilization of triacylglycerol

and glycogen during appressorium turgor generation by Magnaporthe grisea. Plant Cell 12:1703–1718

Taylor SS (1989) cAMP-dependent protein kinase. J Biol Chem 264:8443–8446

Thomas K. Mitchell and Ralph A. Dean(1995) The cAMP-Dependent Pmtein Kinase Catalytic Subunit 1s Required for Appressorium Formation and Pathogenesis by the Rice Blast Pathogen Magnaporthe grisea The Plant Cell,Vol. 7, 1869-1878.

Toda, T., Cameron, S., Sass, P., Zoller, M., and Wigler, M. (1987).Three different genes in S. cerevisiae encode the catalytic subunits of the cAMP-dependent protein kinase. Cell 50, 277-287.

Taylor, S.S., Zheng, J., RadzioAndzelm, E., Knighton, D. R., Eyck, L.F.T., Sowdaski, J.M., Herberg, F.W., and Yonemoto, W.M. (1993). cAMP-dependent protein kinase defines a family of enzymes. Philos. Trans. R. SOC. Lond. B Biol. Sci. 340, 315-324.

Wiederstein M, Sippl MJ. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic Acids Res 2007;35:W407-W410.

Wallner B, Elofsson A. Can correct protein models be identified? Protein Sci 2003;12:1073-1086.

Laskowski RA, MacArthur MW, Moss DS, Thornton JM. PROCHECK: a program to check the stereochemical quality of protein structures. J Appl Cryst1993;26:283-291.

Colovos C, Yeates TO. Verification of protein structures: patterns of nonbonded atomic interactions. Protein Sci 1993;2:1511-1519.

Dong Xu and Yang Zhang. Improving the Physical Realism and Structural Accuracy of Protein Models by a Two-Step Atomic-Level Energy Minimization. Biophysical Journal 2011; 2525–2534.

Verdonk ML, Cole JC, Watson P, Gillet V, Willett P. SuperStar: improved knowledge- based interaction fields for protein binding sites. J Mol Biol. 2001 Mar ;307(3):841-59.

Noel M O'Boyle, Michael Banck, Craig A James, Chris Morley, Tim Vandermeersch and Geoffrey R Hutchison.(2011). Open Babel: An open chemical toolbox. Journal of Cheminformatics, 3:33.

T. Andrew Binkowski, ShaporNaghibzadeh and Jie Liang. (2003). CASTp: Computed Atlas of Surface Topography of proteins. Nucleic Acids Research, Vol. 31, 3352–3355.

Wang Y, Xiao J, Suzek TO, Zhang J, Wang J, Bryant SH. PubChem: a public information system for analyzing bioactivities of small molecules. Nucleic Acids Res 2009; 37: W623-W633.

Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, et al. AutoDock4 and AutoDockTools4: automateddocking with selective receptor flexibility. J Comput Chem 2009;30:2785-2791.

Xuan-Yu Meng1,2, Hong-Xing Zhang1, Mihaly Mezei3, and Meng Cui2. Molecular Docking: A powerful approach for structure-based drug discovery. CurrComput Aided Drug Des. 2011; 7(2): 146–157.

Wallace AC, Laskowski RA, Thornton JM. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein Eng1995;8:127-134.

TatsuyaTamaoki . [28] use and specificity of staurosporine,UCN-01, and calphostin c as protein kinase inhibitors. Methods in enzymology. 2004 jan ;201:340-347.

Hiroyoshi Hidaka and Ryoji Kobayashi. pharmacology of protein kinase inhibitors. Pharmacol. 1992; 32:377-97