International Journal of Scientific Research in Biological Sciences
Vol.5, Issue.4, pp. 119-124, August (2018)
Research Paper
E-ISSN: 2347-7520

Homology modeling and molecular docking studies of Purple acid Phosphatase from *Setaria italica* (Foxtail millet)

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Available online at: www.isroset.org

Accepted: 14/Aug/2018, Online: 30/Aug/ 2018

***Abstract*—**Purple acid Phosphatases (PAPs) hydrolyze insoluble organophosphate compounds to soluble Phosphorous which can be directly acquired by plants from the soil. PAPs have major functions in plant phosphorous nutrition and various studies have been carried out on expression and activity induced by phosphorous starvation. In the present study, the three-dimensional structure of PAPs of *Setaria italica* (Foxtail millet) was generated by homology modeling and was scrutinized by assessment analysis to confirm the suitability and reliability of the predicted model. Further, a molecular docking analysis was carried out using organophosphate compounds such as Diphosphate and Zoledronic acid to study the binding ability. The docked complexes showed the binding affinity of PAPs with the given organophosphate compounds (Diphosphate and Zoledronic acid) possessing energy values of -6.85 and -3.74 Kcal/mol respectively.

Keywords—*PAPs, Homology modeling, Molecular docking, organophosphate, starvation*

I. INTRODUCTION

Foxtail millet (*Setaria italica*) belonging to *Panicoideae* subfamily, was domesticated from the wild species, green foxtail (*Setaria viridis*) more than 8000 years ago in Northern China [1]. It remains an important cereal crop in arid and semi-arid regions of China and India. Two different foxtail millet reference genomes are available [2, 3]. Comparative genome analysis revealed a high level of collinearity between foxtail millet and rice (*Oryza sativa*) [4] In addition, foxtail millet has been proposed recently as a novel model species for functional genomics studies of the Panicoideae because of its small diploid genome (2n=18, ~510Mb), short life cycle, small stature, prolific seed production and C4 photosynthesis [5, 6]. Plant growth and development require macro and micronutrients, some of which are available only from external sources like the soil in which they grow. Phosphorous (P) is one such essential macronutrient for plant growth and development [7]. Many soils are deficient in phosphate which results in phosphate starvation for plants growing in them. Plants develop several strategies to overcome phosphate limitation, including morphological, biochemical and physiological responses to enhance the acquisition of this vital macronutrient. These combined responses enhance the chances of plants to survive the prospects of phosphate starvation. Due to continuous leaching, ancient soils are known to become limited in the availability of phosphate.

Although P is an essential macronutrient, it cannot be directly utilized by plants [8]. It has been estimated that 30–65% of total P in soils is present as organic P, mainly in the form of phytate, nucleic acids and phospholipids [9, 10]. Phosphorus is a key component of nucleic acids and phospholipids. Inorganic phosphate (Pi) and organophosphates are the major forms of P acquired by plants from soil directly [11]. Low Pi availability is a major constraint limiting plant growth and production on both natural and agricultural soils [12, 13]. Plant roots readily uptake the required amount of hydrolyzed form of P which has higher absorption capacity and which varies from one plant to the other. Therefore, the capacity of plants to find an adequate phosphate supply is directly correlated with their ability to explore the soil [14].

Purple acid phosphatases (PAPs) belong to the family of dinuclear metallohydrolases which are characterized by the purple/pink colour of protein in water solution and carry out a hydrolysis reaction [15, 16]. PAPs have major function in plant P nutrition and they are induced by P starvation [17, 18, 19]. Secreted PAPs are likely to get involved in conversion of insoluble organophosphate compounds to soluble P form in the rhizosphere with optimal activity at pH below 7.0, whereas remobilization and recycle of P from intracellular P reserves in plants under P starvation conditions are carried out by intracellular PAPs [18, 20].

PAPs from plant species like soybean, sweet potato and *Arabidopsis* have been purified and their amino acid sequences were elucidated [22, 23, 24]. This helps in the identification of sequence patterns of PAPs and identification of a large number of novel PAP and PAP-like sequences in plants [24]. Furthermore, with the availability of genomic sequences for diverse plant species, PAP members have now been widely identified and characterized in plants such as *Arabidopsis* [25], soybean [26] and maize [9,27,28]. Biochemical analysis of plant PAPs showed that there is a wide range of phosphorylated compounds both natural and synthetic, such as ρ-nitrophenyl phosphate, energetic compounds (ATP and inorganic pyrophosphate), phosphorylated sugars and phosphorylated amino acids [22 ,23]. Due to the lack of crystallographic structure of PAPs, the present investigation deals with homology based prediction of three dimensional structure of PAP from *Setaria italica* and structural characterization was done for the same. Further Molecular docking was carried out to understand its binding ability with organic phosphates (Diphosphate and Zoledronic acid) as PAPs were shown to have their binding affinity with other phosphorylated compounds.

II. METHODOLOGY

Determination of physicochemical parameters of the target protein

Amino acid sequence of purple acid phosphatase from *Setaria italica* was retrieved from the Phytozome genome database (Reference) using Arabidopsis purple acid phosphatase sequence AT5G34850 (PAP26) as template. The physical and chemical property analysis is an important step in protein structure prediction which invariably affects the structural stability and the functional interaction of the protein in the cellular milieu. The physico-chemical parameters of the enzyme purple acid phosphatase from *Setaria italica* was analyzed by ExPASy ProtParam tool (http://web.expasy.org/protparam) [29]. Physicochemical parameters like molecular weight, PI, Instability index, aliphatic index, grand average hydropathy (GRAVY) etc., were calculated. The calculated PI helps in determining isoelectric point of the protein whereas GRAVY score infers the interaction pattern of the protein with the water molecules.

Analysis of functional motifs

The target sequence was analyzed for the putative functional motifs using MOTIF finder (http://www.genome.jp/tools/motif/). This predicts the probable functional motifs within the protein with a statistically significant cut-off.

Generation of three dimensional structures of protein and its validation

The overall secondary structure of purple acid phosphatase was analyzed using PSIPRED 4 [30]. The amino acid sequence was subjected to BLASTp against Protein Data Bank (PDB) for selection of templates. The top four templates were used for the prediction of 3D structure of purple acid phosphatase by Modeller v9.18 [31]. Loop modeling and subsequent energy minimization was performed using Swiss-PDB Viewer [32] and validated by RAMPAGE [33].

Docking simulations

Chemical structure of Diphosphate and Zoledronic acid were downloaded from PUBCHEM compound database. The structures were converted to PDB format using OpenBabel [34]. The docking process was carried out using Auto dock tool [35]. As the searching grid extended above the preferred target proteins, polar hydrogens were added to the ligand moieties. Kollman charges were assigned and atomic solvation parameters were added. Polar hydrogen charges of the Gasteiger-type were assigned and the nonpolar hydrogens were merged with the carbons and the internal degrees of freedom and torsions were set. The compounds were docked to the target protein with the molecule considered as a rigid body and the ligands being flexible. The search was extended over the whole receptor protein used as blind docking. Affinity maps for all the atom types were present and an electrostatic map also was computed with grid spacing of 0.375Å. The search was carried out with the Lamarckian Genetic Algorithm; populations of 150 individuals with a mutation rate of 0.02 were evolved for 10 generations. Evaluation of the results was done by sorting the different complexes with respect to the predicted binding energy. A cluster analysis based on root mean square deviation values with reference to the starting geometry was subsequently performed and the lowest energy conformation of the more populated cluster was considered as the most trustable solution. The hydrophobic effect of ligand was retrieved by ALOGPS 2.1. This Applet provides interactive on-line prediction of logP, water solubility and pKa(s) of compounds for environmental chemistry studies. The receptor–ligand complexes were analyzed using Discovery Studio Visualizer v17.2.0.16349 (BIOVIA, Wateridge Vista Drive, San Diego, CA, USA).

III. RESULTS AND DISCUSSION

The Purple Acid Phosphatases (PAPs) are said to play a role in phosphate scavenging when cells are depleted or starved of phosphate and under oxidative stress. Thus PAPs are crucial for the cellular metabolism and energy transduction processes. Analysis of physicochemical properties of *Setaria italica* PAP showed that the target protein represents the exact molecular weight of 55028.49 Da (Table 1). The calculated PI (6.68) revealed that the protein is acidic in nature. Instability index of a protein confirmed the instability of the protein in the cellular environment. It is also helpful in delineating the half-life of a protein. Theoretically, the proteins with a half-life ≤ 5 hours are not very much stable

and exhibits higher instability index (≥ 40) and those having greater stability confer lower instability index (≤39) [36, 37]. The enzyme exhibited an instability index of 44.76, depicting that the enzyme was not very much stable in cellular environment but may exhibit moderate stability instead. The aliphatic index demonstrated the relative amount of aliphatic side chains (Alanine, Valine, Isoleucine, Leucine). It may be regarded as a positive factor for the increase of thermo stability of globular proteins [38]. The aliphatic index of the protein was found to be 61.65. The most important parameter among the physico-chemical properties was grand average hydropathy (GRAVY) score, which was calculated by adding the hydropathy values of each amino acid residues and making average with the length of the sequence [39]. GRAVY score of the protein was <-0.4, which indicated its hydrophilic nature. The total number of positively and negatively charged residues present in the protein was about 48 and 40 respectively.

Analysis of functional motifs through MOTIF finder unveiled the presence of three metallophosphoesterase domain, a single purple acid phosphatase and a domain of unknown function. Purple acid phosphatase was represented by 19 to 109 amino acid residues. Metallophosphoesterase was located in between 120 and 317 amino acids. A domain with unknown function was found to be in between 216 and 276 amino acid position (Figure 1a and Table 2). The secondary structure of the target protein predicted by PSIPRED 4 is given in Figure 1b. Multiple sequence alignment with other PDB files revealed best match with A chain of Purple acid phosphatase (PDB IB: 1XZW) with an E-value of e-158.

The BLAST result of PAPs from *Setaria italica* exhibited step similarities with PAPs of kidney bean and sweet potato (PDB IDs: 1KBP, 1XZW, 2QFP and 4DSY) with the alignment scores >500. Based on this we used 1KBP, 1XZW, 2QFP and 4DSY as templates which showed sequence identity of 60%, 61%, 61% and 61% respectively. All the templates were further aligned with the target in order to predict the structure of the target protein using Modeller tool. 10 models were generated and the best model was chosen based on DOPE (Discrete Optimized Protein Energy) score.

Among this Model1 was found to be the best model and was subjected for loop modeling. Validation of the structure using RAMPAGE server exhibited 95.2% residues in favored region, 4.8% in allowed region and no residues in outlier position (Figure 2b). . The protein structure was further visualized using PyMOL (Figure 2a). The binding of Hydrobond distance for ARG`261was 2.012 and ALA`218 was 1.935. PAP when docked with the Diphosphate showed the prime interactions with SER` 417, ARG` 321, SER` 319, ARG` 318 and ARG` 419 (-6.85 kcal/mol) in this ARG` 318 showed hydrogen bond distance of 1.675 (Table 3). The hydrogen bonding between PAP and Zoledronic acid is shown with TYR` 286, ASN` 280, GLU` 290, ARG` 293, ASN` 324 and ARG`334. The hydrogen bond distance was 2.016 for ASN` 280, 2.166 for TYR 286, 1.995 for ARG` 293 and 2.011 for ASN` 324 (-3.74 kcal/mol) (Figure 3 and Figure 4).

The PAP enzymes may exist in two forms: the inactive purple form, where the redox-active iron is in the ferric state and the active pink form, where the iron is reduced to the ferrous state. The PAPs are expressed in a tissue specific manner and often exist as isozymes [40, 41]. The isozymes exhibit variation in molecular structure, substrate specificity and localization. Some PAPs, such as alkaline peroxidase / purple acid phosphatase from *Solanum lycopersicum* are multifunctional enzymes that have both a purple acid phosphatase and an alkaline peroxidase function. These enzymes are hypothesized to function in the production of reactive oxygen species (ROS) [42]. The phosphatase and oxygen radical-generating activities of PAPs are functionally independent and similar to those of mammalian PAP [43]. Once absorbed, the phosphate is utilized inside the cells at optimum cellular concentration for the various developmental and biochemical processes.

Phosphate acquisition is a complicated process and has various modified routes. The phosphate homeostasis is maintained by acquisition, limited consumption and effective recycling, when the supply and demand is not equitable. The molecular basis for these strategies is being studied in a number of species [44]. The Gulf Ryegrass is an ideal model plant as it hyper accumulates phosphate utilizing multiple acquisition and mobilization systems under varying phosphate regimes [45]. In addition to its biologically diverse functions, PAP is also catalytically promiscuous; not only the enzyme is able to operate as a mono- and diesterase, but also some PAPs are also bifunctional with both hydrolytic and peroxidase activities.

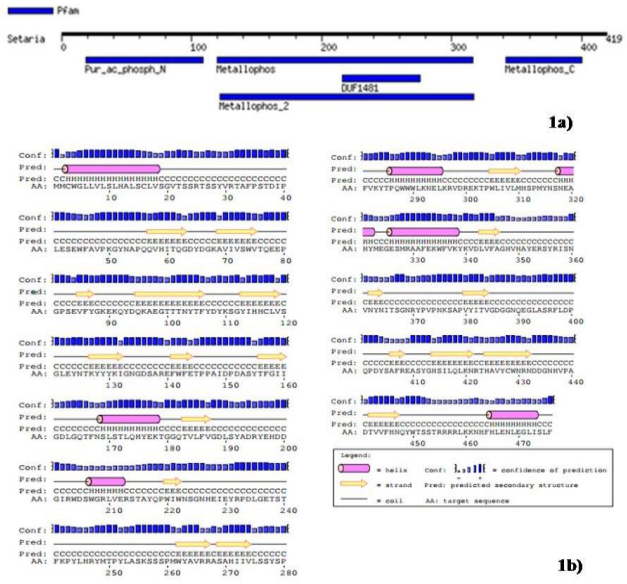
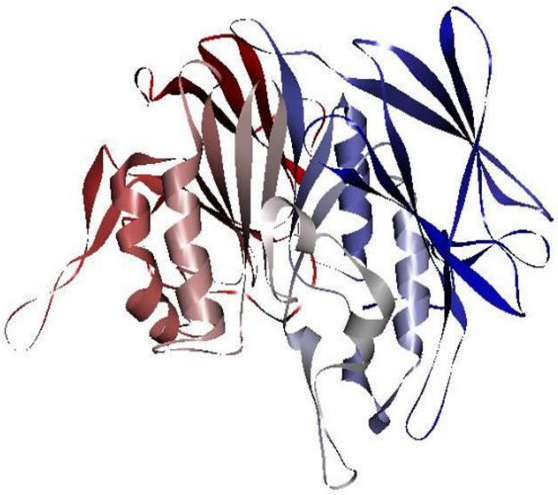
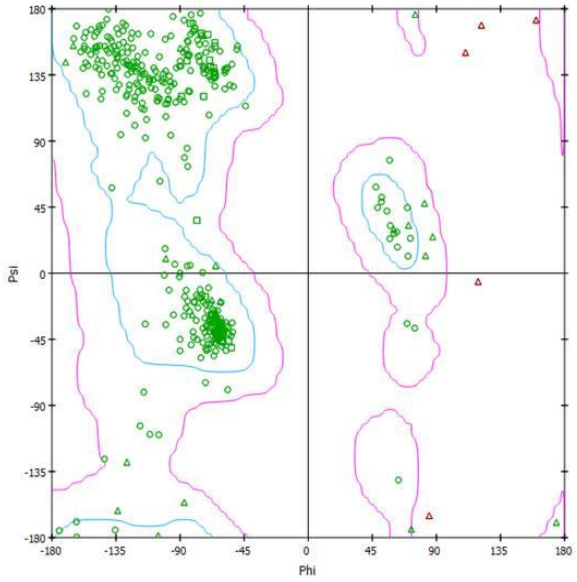


Figure 1a) Functional and 1b) Secondary structure of purple acid phosphatase (PAPs) from Setaria italica.



2a)



2b)

Figure 2a) Predicted 3D structure and 2b) Ramachandran Plots for 3D structure of purple acid phosphatase (PAPs) from Setaria italica.

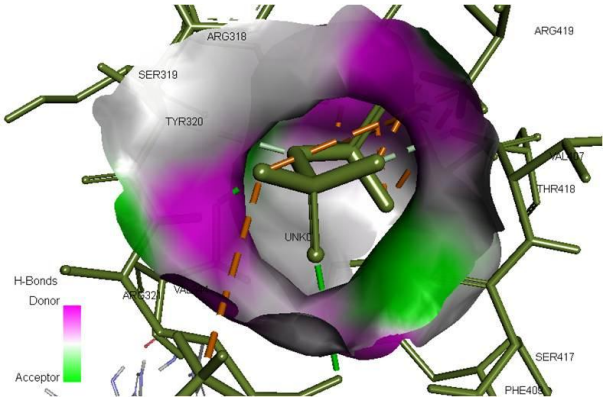


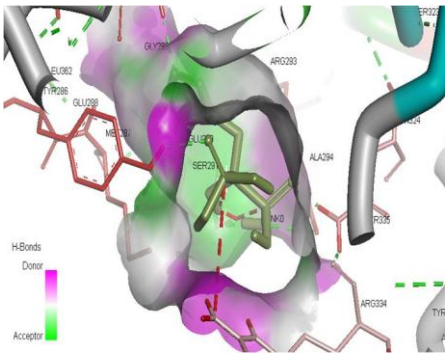
Figure 3. Maps of hydrogen bonds at the binding site of purple acid phosphates of Setaria italica docked with Ligand Diphosphate.

Table 1. Physical and Chemical parameters of *Setaria italica* Purple Acid Phosphatase (PAPs)

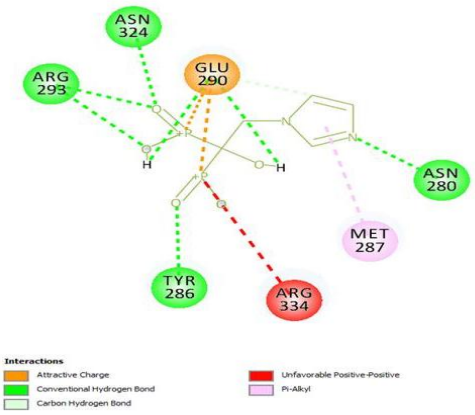
S. No	Parameter	Value
1	Number of amino acids	419
2	Molecular weight	48581.87
3	Number of negatively charged residues	48
4	Number of positively charged residues	40
5	Theoretical pI	6.05
6	Instability index	42.26
7	Aliphatic index	61.65
8	GRAVY	-0.682

Table 2. Conserved motifs in *Setaria italica* Purple Acid Phosphatase (PAPs)

S. No	Pfam	Position	E value	Description
1	Metallophos	120-316	1.7e-21	Calcineurin-like phosphoesterase
2	Pur_ac_phosph_N	19-109	1.1e-20	Purple acid phosphatase
3	Metallophos_C	342-400	2.6e-16	Iron/zinc purple acid phosphatase-like protein C
4	DUF1481	216-276	0.91	Protein of unknown function
5	Metallophos_2	122-317	0.53	Calcineurin-like phosphoesterase superfamily domain



4a)



4b)

Figure 4. Maps of hydrogen bonds at the binding site of purple acid phosphates of Setaria italica docked with Ligand Zoledronic acid; B) Interaction diagram for PAPs– Zoledronic acid complex.

Table 4. Energy and RMSD values obtained during docking analysis of selected Ligand molecules and PAPs from *Setaria italica* as target protein

Protein	Ligand	Interacting molecules	Binding Energy	Vdw_hb_desolv_enegry	Electrostatic Energy	Inhibition constant
Purple Acid Phosphatase	Disphosphate	SER` 417/O with 1 atoms; ARG` 321/HN with 1 atoms; SER` 319/O with 1 atoms; ARG`318/2HH1 with 1 atoms; ARG` 419/HN1 with 1 atoms; ARG` 419/HN2 with 3 atoms	-6.85	-2.7	-4.74	9.58 µM
	Zoledronic acid	TRY`286/HH with 1 atoms; ASN` 280/O with 1 atoms; GLU`290/OE2 with 2 atoms; ARG` 293/2HH1 with 6 atoms; ASN` 324/1HD2 with 2 atoms; ARG` 334/O with 1 atoms	-3.74	-5.43	-1.0	1.81 mM

IV. CONCLUSION AND FUTURE SCOPE

PAPs form a genetically diverse large group of di-nuclear metallohydrolase that are involved in a multitude of biological roles. In the present study we have successfully predicted the molecular 3D structure of *Si*PAPs and also found the binding ability of PAPs using commonly available organophosphates. This study proved that the PAPs have strong binding ability with phosphorus esterase forms that can be converted into organic phosphorus form which the plant can easily uptake during phosphate deficiency. Over all this study proved the functional importance of PAPs under stress condition. Factors that contribute towards this functional and mechanistic versatility of PAP are still poorly understood. Thus, despite significant efforts and advances, PAP still poses considerable challenges for inorganic and biological chemistry.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

The authors wish to thank the Bioinformatics Division, Department of Biotechnology, Ministry of Science and Technology, Government of India for extending financial support through Bioinformatics Infrastructure Facility for BTISnet program.

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