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Novel insight into the molecular interaction of catalase and sucrose: A combination of *in silico* and *in planta assays* study

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ABSTRACT

Osmolytes are known to be an important factor for the stabilization and proficient functioning of proteins. However, the stabilization mechanism of proteins by the interaction of osmolytes is still not well explored. Here, we performed *in silico* 3D structure modelling of rice catalase-A (CatA) protein and its molecular interaction with sucrose. Further, *in planta* was conducted to see the effects of sucrose on catalase activity in rice grown in saline sodic soil at different time intervals. The molecular docking experiments results showed that sucrose can be ligated with CatA, protein forming hydrogen bond with precise amino acid residues like, R49, R89, P309, F311, Y335 and T338. The interaction also comprises the contribution of hydrophobic amino acid residues like V50, V51, H52, L123, A310, Q339 and R342. The *planta in vitro* catalase activity assay showed that plants treated with sucrose significantly affect the catalase activity in rice. Results revealed that maximum catalase activity was recorded in plants treated with 150 and 200 ppm of sucrose after 15 days of sucrose application. However, minimum activity was recorded in control plants. We believe that our study will provides an advanced understanding of catalase activity in plants exposed to osmotic stress.

1. Introduction

In living organisms, the interaction of carbohydrates with protein play key role in a wide range of biochemical processes that remain linked to hormones, enzyme, antibodies etc. having importance in the field of immunology, bio-synthesis, pharmacology and medicine (Metzler, 2003). These interactions also hold importance in plant response to biotic and abiotic stress, salt-stress in particular.

Salinity in the form of both hyper-osmotic and hyper-ionic stresses imparts a major threat toward crop productivity. Extensive research has been conducted to investigate the molecular mechanism for control of this stress factor (Arora et al., 2002; Foyer and Noctor, 2000; Mittler, 2002). It has been proven that salt can induce oxidative stress (Scandalios, 2005) leading to accumulation of Hydrogen peroxide (H_2O_2) in the cell organelles. H_2O_2, relatively a stable oxy-molecule if remain un-scavenged can also become deleterious to plant metabolic activity as the peroxide can undergo chemical modification producing other highly reactive oxy-radicals that can react with cellular constituents like lipids, proteins, nucleic acids etc. (Foyer and Noctor, 2003). In order to overcome the deleterious effect of H_2O_2, plants in coordination with several non-enzymatic scavengers (ascorbate, tocopherol, carotenoids etc.) also employs a group of enzymatic scavengers like catalase, peroxidase and other substrate specific peroxidases (ascorbate peroxidase, glutathione peroxidase etc.) to scavenge H_2O_2 in order to maintain the cellular concentration of peroxide to a level having its physiological significance (Willekens et al., 1997).

Among the various H_2O_2 scavenging enzymes, catalase having a low affinity for its substrate (high *Km*) but with significantly high processing rate (conversion of H_2O_2 to H_2O and O_2) plays a central protective role against oxidative stress developed by salt-induced accumulation of H_2O_2. However, literature reports suggest that catalase is highly susceptible to salt-stress where its activity is down regulated (Cavalcanti et al., 2004; Lee et al., 2001; Nishikawa et al., 2004; Shim et al., 2003; Valluru and Van den Ende, 2008). As expected Sahu et al. (2010) have shown a declined rice leaf catalase activity under elevated salt-treatment, however, surprisingly, supplementation of sucrose significantly

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rescues salt mediated decay in catalase activity. The investigation also evidenced that catalase in rice leaf interacts with sucrose under *in vivo* condition and renders a protective role in maintaining high enzymatic activity thus acting as the principal enzymatic source for scavenging the stress induced production of H_2O_2 (Sahu et al., 2010).

Although, the exact mechanism of interaction of sucrose with enzyme protein(s) is yet to be deciphered, it has been conclusively shown that sucrose can substantially protect the inactivation of enzymatic activity of many proteins during stress insult to organisms (García et al., 2007). In order to get some further insight into the interaction of sucrose with catalase an attempt has been made here to develop a 3D *in silico* model of rice Cat-A and the study has been extended to examine the interaction of sucrose with the enzyme protein. The best validated model was further subjected to molecular dynamics simulation to study dynamic behavior of the proteins. Further, effects of sucrose on CatA activity were evaluated in rice under saline sodic soil.

2. Material & method

2.1. Domain architecture of catalase-A (Cat-A)

The protein sequence of CatA was reported earlier, and retrieved from the NCBI (ID: ABN71233) for the present investigation. The functional domains of Cat-A protein were inferred using Conserved Domain Search Service (CD Search) (Marchler-Bauer et al., 2016), Pfam database (Finn et al., 2016) and Simple Modular Architecture Reseach Tool (SMART) (Letunic et al., 2014). ProtParam predicted various physio-chemical properties of Cat-A.

2.2. 3D structure Generation, refinement and validation

It was verified that the three-dimensional (3D) structure of the CatA protein has not yet reported in the protein data bank (PDB). Therefore, we developed the 3D model of CatA protein. BLASTP (Altschull et al., 1990) search was performed against PDB (Berman et al., 2006) to find the appropriate homologous templates for structure modelling. CatF (PDB code: 1m7s) was showing best homology with target sequence. The sequence identity was found to be 43 per cent between the template (1m7s) and (CatA) target sequence. The ClustalW (http://www.ebi.ac.uk/clustalw) (Thompson et al., 1994) program was applied for sequence alignment between CatA and 1m7s.

MODELLER (http//:www.salilab.org/MODELLER) (Šali and Blundell, 1993) was used for the development of 3D structure. The MODELLER utilizes 'probability density functions' as the spatial restraints (Sali et al., 1993; Šali and Overington, 1994). In MODELLER, python script with automodel class was used for constructing the models (Eswar et al., 2006). Total twenty models of 3D structure were generated and later discrete optimized protein energy (DOPE) scores were calculated for each model using normalized DOPE assessment method available in MODELLER. DOPE score allots a score for each model by scrutinizing the positions of all non-hydrogen atoms, with lower scores predicting more accurate models. We also calculated Ramachandran plot for all the 20 models and based on maximum residues in the favored region and less in disallowed region were selected for further course of action. Finally, CatA models with lowest DOPE value were validated by PROCHECK (Laskowski et al., 1993) and VERIFY3D (Eisenberg et al., 1997) and ERRAT was further subjected to energy minimization using CHARMm (Brooks et al., 1983) force field. The energy-optimized models of CatA were verified for stereo-chemical quality using SAVeS server (http://nihserver.mbi.ucla.edu/SAVES/). ProSA-web (Wiederstein and Sippl, 2007) was used to calculate the energy potential of the model. This model was further used for docking studies with Sucrose using GOLD docking program.

2.3. Molecular dynamics simulations

To study the dynamic properties, MD simulation for the protein–ligand complex (catalase-sucrose) was carried out with the GROMACS 4.6.4 (Hess et al., 1997) package using the GROMOS96 54a7 (Oostenbrink et al., 2004) force field. The conformation with lowest binding energy was used as the initial conformation for MD simulations. First the topology of the protein was created by using the Gromacs program while for creating sucrose topology was created using PRODRG (http://davapc1.bioch.dundee.ac.uk/cgi-bin/prodrg). The complex was immersed in dodecahedron box of simple point charge (SPC) water molecules. The solvated system was neutralized by adding counter-ions. Energy Minimization of the solvated structures was done using steepest descent and conjugate gradient algorithm till maximum force reached below 100KJ/mol/nm. To equilibrate, the system was subsequently subjected to the position-restrained dynamics simulation (NVT and NPT) at 300 K for 100 ps. Finally, this system was subjected to MD production run for 100 ps at 300 K temperature and 1 bar pressure as our previous work (Tandon et al., 2015).

For trajectory analysis, various parameters were computed using GROMACS. These included Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF) and Potential energy. Principal Component Analysis (PCA) was also performed to explore the global motion of the coordinates of atoms of the Catalase-Sucrose complex during MD Simulations. PCA can characterize the cumulative and overall motion of the system. PCA calculations were examined by using g_covar and g_aneig functions of GROMACS. Our analysis was restricted to Cα atoms, as it is less disturbed by statistical noise.

2.4. Study of protein – ligand interaction

The structure of sucrose molecule was fetched from pubchem (PubChem CID:5988) (http://pubchem.ncbi.nlm.nih.gov) in 2D-strucuture data file (SDF) format and converted into 3D-Mol2 file with the program CORINA2.6 (Hagler et al., 1974; Tetko et al., 2005). Energy minimization and ligand preparation was further performed using Discovery studio suit. Ten pockets were recognized on the modelled 3D structure of CatA protein using Q Site-Finder tool. Molecular docking study was executed using GOLD (Genetic Optimization Ligand Docking) Software. The docking parameters were set with reference to earlier studies (Afiqah et al., 2016; Fazil et al., 2012). GOLD uses a genetic algorithm (GA) to explore the full range of ligand flexibility of conformation with partial flexibility of the protein (Jones et al., 1997). The binding affinity was also estimated by using the consensus scoring function X-Score V2.1 (Wang et al., 2002). LIGPLOT (Wallace et al., 1995) was used to show 2d binding of protein ligand complex. GETNEARES tool (available with the program DOCK) was applied to double-check the hydrogen bond interactions (Ewing et al., 2001).

2.5. In planta assay

2.5.1. Planting material, growth conditions and experimental design

Rice seeds (*cv.* Pusa Basmati 1) were obtained from Farm Section, ICAR-Indian Institute of Seed Science, Kushmaur, Maunath Bhanjan, U.P., India. Seeds were planted in pots under nethouse conditions. Each pot containing 5 kg of experimental soil (pre-sterilized). The basic property of soil is saline sodic with pH 8.2 and EC 1.82. Experiments were conducted during August - September with relative humidity of 90% and average mean temperature of 31.25 °C.

The experimental design consisted of five different treatments where plants were treated with different concentration of sucrose (50, 100, 150 and 200 ppm) and absolute control (untreated). Ten seeds were sown in each pot. The moisture content in the pots was kept at field capacity (60%) by sprinkling with sterilized water on every alternate day. After thirty days of sowing, plants were treated with different concentration of sucrose solution at 5 ml per plant in the evening