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Homology Modelling Of Auxin Induced Glutathione S-Transferases from *Prosopis Juliflora*

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Abstract: *Prosopis juliflora (Swartz) DC, fast growing, nitrogen-fixing and tolerant to arid conditions and saline soils. Glutathione S-transferases (GSTs) play roles in both normal cellular metabolisms as well as in the detoxification of a wide variety of xenobiotic compounds, and they have been intensively studied with regard to herbicide detoxification in plants. The present study deduced the homology modeling of target sequence from Prosopis Juliflora (gi|189031607|gb|ACD74942.1|) and obtain best template protein sequence (3MOF) from Protein Data Bank. The amino acid primary structure analyses done by using "Bio-edit" and Secondary structure prediction validate by using chimera and Ramachandran plot. The bioinformatics tools have been used to identify best cavity 6.8 with 13 sites to binding GSH ligand. The proposed model further explore for in In-Silico docking with Argus lab engine with minimum energy; -9.85288 k/mol and further validated with help of Hex dock engine with fitness electrostatics calculation -257.64 and web server ME-Dock for maximum entropy based docking found 7.47857 kcl/mol. The result obtain form different In-Silico tools for dock GSH ligand into template protein are useful in homology modeling, simulation and structural based virtual screening for novel drug designing.*

Keyword: *Active site, Glutathione S-transferases, homology modeling In-Silico docking, Prosopis juliflora*

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I. Introduction

Prosopis juliflora fast growing, nitrogen-fixing and tolerant to arid conditions and saline soils. In India sub-continent, it is exotic tree species and introduced during 1877 from native range Central America and northern South America to India. Resulted, it is well adapted and spread through India.[1] Under the right conditions, *Prosopis juliflora* has survived where other tree species have failed. [2] In 2004 it was rated one of the world's top 100 least wanted species (Invasive Species Specialist Group of the IUCN, 2004).[3]

Glutathione S-transferases (GSTs) play roles in both normal cellular metabolisms as well as in the detoxification of a wide variety of xenobiotic compounds, and they have been intensively studied with regard to herbicide detoxification in plants. [4,5] A newly discovered plant GST subclass has been implicated in numerous stress responses, including those arising from pathogen attack, oxidative stress, and heavy-metal toxicity. In addition, plant GSTs play a role in the cellular response to auxins and during the normal metabolism of plant secondary products like anthocyanins and cinnamic acid and GST gene from *Prosopis juliflora,* was transformed into the tobacco and Indica rice variety ADT-43 the stress studies revealed protective function of this gene under different a-biotic stresses. [6] The transgenic plants survived better under conditions of abiotic stress compared to control. In plant Glutathione (GSH) ($C_{10}H_{17}N_3O_6S$) molar mass (307.32 g/mol) is a tri-peptide that contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side-chain. [7] It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. The IUPAC name (2*S*)-2-amino-4-{[(1*R*)-1-[(carboxymethyl)carbamoyl]-2-sulfanylethyl]carbamoyl}butanoic acid. GSH is found almost exclusively in its reduced form, since the enzyme that reverts it from its oxidized form, glutathione reductase, is constitutively active and inducible upon oxidative stress. In fact, the ratio of reduced glutathione to oxidized glutathione within cells is often used as a measure of cellular toxicity. In plants, glutathione is crucial for biotic and abiotic stress management. It is a pivotal component of the glutathione-ascorbate cycle, a system that reduces poisonous hydrogen peroxide; it is the precursor of phytochelatins, glutathione oligomeres that chelate heavy metals such as cadmium. Glutathione is required for efficient defense against plant pathogens such as *Pseudomonas syringae* and phytophthora brassicae. [8] APS reductase, an enzyme of the sulfur assimilation pathway uses glutathione as electron donor. Other enzymes using glutathione as substrate are glutaredoxin, these small oxidoreductases are involved in flower development, salicylic acid and plant defence signalling.

II. Material and Method

The amino acid sequence of auxin induced Glutathione S-transferae (GSTs) was extract 19 Amino acid sequence from NCBI- ***(http://www.ncbi.nlm..nih.gov)*** and later submitted to Swiss Port Database, homology searched against target sequence (aca43742.1). [9] Protein Data Bank (http://rcsb.org/pdb/), extract suitable 44 templates for the query sequence filter by 99% similarity viz. (Table-1). These templates sequences are closed reference to candidate templates PDB ID (3mof) with length found to be 624 long amino acid and source of the template is *rattus norvegicus* (http://rcsb.org/pdb. **[10]** The primary properties of protein sequence was analyzed by using BioEdit tool. [11] Protein secondary structure carried out using Chimara tool and the same was employed in three dimensional structures.[12] To correct the geometric inaccuracies the theoretical model was subject to Procheck **[13]** The protein ligand interaction was performed using Argus Lab engine with flexible docking and calculate binding cavity using active site prediction server (online tools). [14] Docking analysis was carried out using online programme viz. Medock and Hex 6.3. [15,16,17]

Table 1:- FASTA results from a search of the sequence of PDB entry 3mof (A) against all protein sequences in the PDB.

PDB ID	Smith-waterman score	% identity	AA overlap	Seq. Length	Z score	E value
3dt7	4316	99.98	624	624	5288.8	0
28k8	4303	99.8	622	622	5272.9	0
3dt2	4296	99.8	621	621	5264.3	0
3dtb	4286	99.8	620	620	5252.0	0

III. Results

The *In-Silico* tools as indicated in the previous section, viz., homology molding, active site analysis and docking were employed in the present study for screening legend to bind to the active site of 3mof. The homology modeling of reference sequence from the protein data bank and final model was evaluated using Procheck. Further validation analysis employing Ramachandran plot reviled that 3mof model showed 91.17% of residues lie in the most favored region allowed of the plot (Fig-1) [18]. The UCSF Chimera for protein structure prediction shows the 3mof protein structure with two side chain(Fig-2) and obtain from online web server of protein active site prediction calculate binding number of 63 cavity in protein (Fig-3).[12]

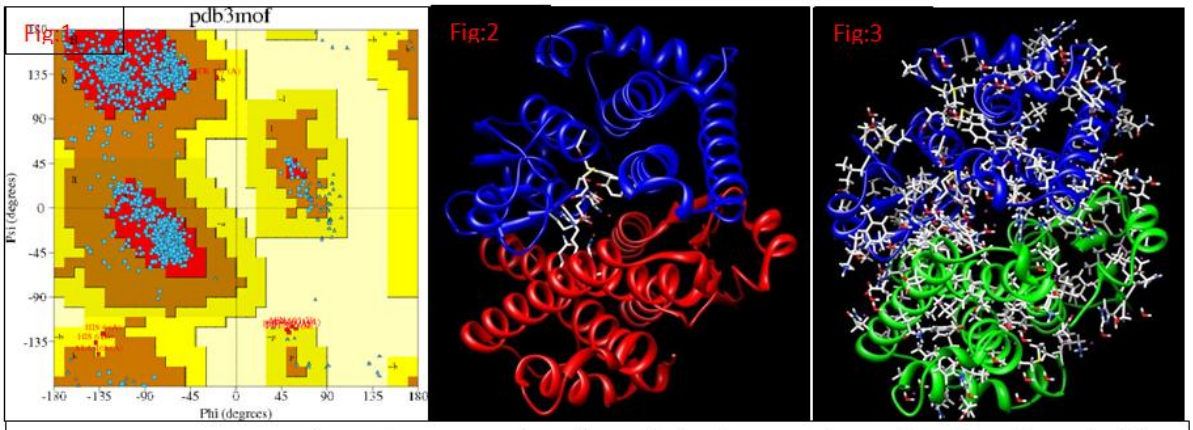

Fig-1: Rmachandran plot showed 91.17% of residues lie in the most favored region allowed of the plot, **Fig-2**: using Chimara tools for protein structure prediction shows the 3mof protein structure with two side chain., **Fig3:** Online tool for Active site prediction of 3mof protein shown 63 cavity.

Table 2:- Out of 63 cavities obtain from active site prediction server best 5 given below

Cluster	Best Cavity (k)
13	6.8
11	6.8
23	6.7
10	5.9
3	5.8

The active site finder identified all cavities in a protein and scores them based on the physicochemical properties of functional groups in protein. The accuracy realized on 620 proteins with sizes ranging from 100 to 600 amino acids. Template protein in Table -2 shown best 5 activity cavity out of 63 cluster and predict that binding site of ligand are one of them.

The docking of template protein with Argus lab engine [14] gave an insight into the binding mode of various ligands. In case of GSH ligand [7] active site resides viz. 220 found with a strong hydrogen bond interaction with 3mof molecule from default parameter (dock engine, dock calculation and ligand algorithm flexible) and least energy -9.85288 kcal/mol with best ligend pose (Fig-4).

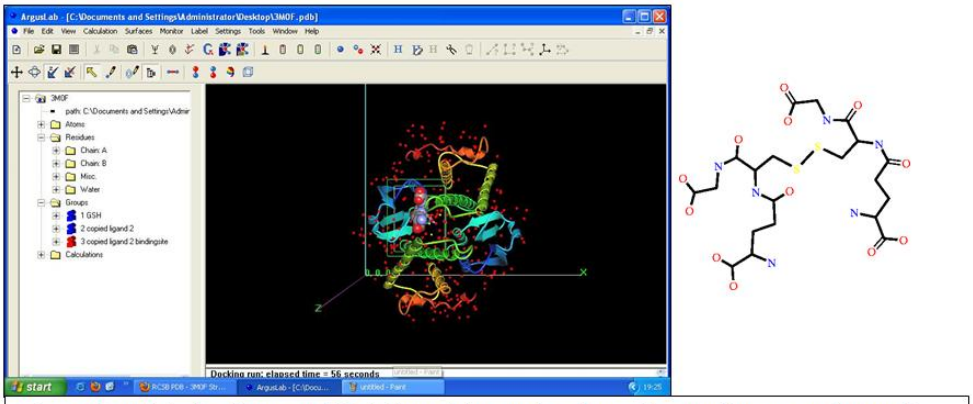

Fig-4: Ligand docked in Argus lab indicated that active site resides position at 220 in 3mof protein. This made a strong hydrogen bond interaction with least energy -9.85288 k/mol. Fig-8: GSH ligand ($(C_{10}H_{17}N_3O_6S)$ molar mass (307.32 g/mol)).

Docking validation using hex dock 6.3. [17] In *Hex* 's docking calculations, each molecule modelled using 3D expansions of real orthogonal spherical polar basis functions to encode both surface shape and electrostatic charge and potential distributions. Essentially, this allows each property to be represented by a vector of coefficients calculates standard control (receptor, ligand range-80 and solution- 2000, step size- 7.5), score fitness found to be electrostatics calculation -257.64 (Fig-5).

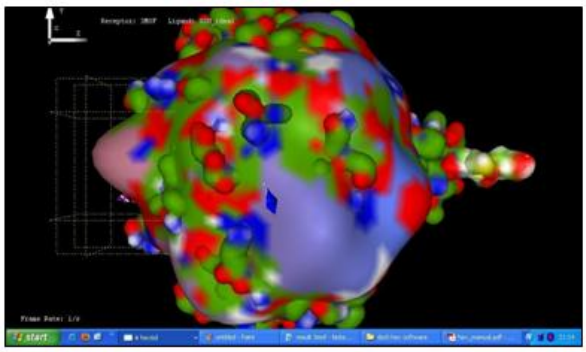

Fig (5) -: Docking in Hex-6.3:- docking study with Hex dock showed fitness electrostatics calculation -257.64 in template protein with bind GSH ligand

In online validation, maximum entropy based docking, the goal of this sever is to provide an efficient utility for predicting ligand binding sites. [19] With the help of ME-Dock web server, incorporates a global search strategy that exploits the maximum entropy property of the Gaussian probability distribution in the context of information theory. [20] Dock setting (dock cycles 5 and population size 50) to calculate maximum entropy result (Table-3). Lowest docking energy found -7.27738 kcl/mol of best cluster in template portein (Fig-6).

Table 3:- Summary of individual docking run in ME-Dock online tools, best energy found -7.48 in 3 cycles (http://mbi.ee.ncku.edu.tw/wiki/doku.php?id=medock)

Rank	**Run (Cycle)**	**Docked Energy** kcl/mol	**Random Seed**
1	3	-7.48	18545
2	1	-7.39	18545
3	5	-7.19	18545
4	4	-7.16	18545
5	2	-7.16	18545

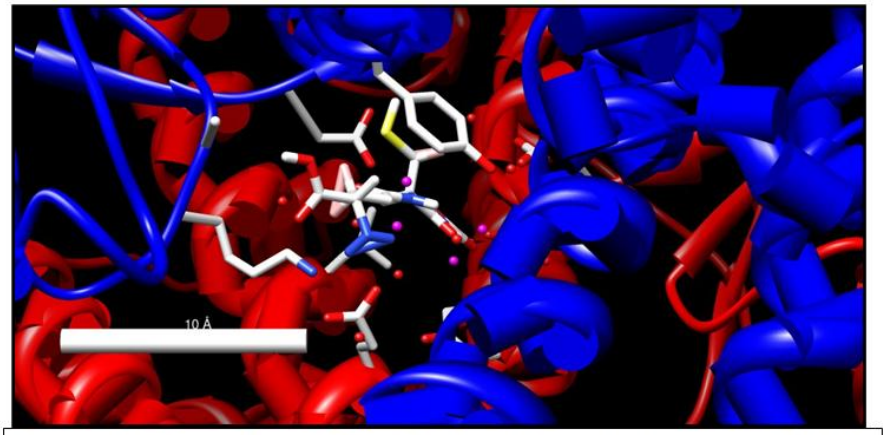

Fig (6) -: Docking study with Me-Dock showed lowest docking energy:7.47857 kcl/mol in template protein with bind GSH ligand

IV. Discussion

The proteomic analysis of 3mof revealed its primary properties and secondary structural information and they are essential to understand it, structure, function and nature of interaction. The stereochemistry evaluation of predicted 3D structure of target template suggested that the proposed model for good quality. The most common goal of protein-ligand docking very important as an inhibitor, anti-cancer drug design. [21, 22] Moreover, major phase of enzymatic detoxification in many species reported that its conjugation of activated xenobiotic to reduced glutathione (GSH) catalyzed by the (GST), usually some compounds, once transformed into GST, enter the mercapturic acid pathway whose end products are highly reactive and toxic for the cell responsible for their production. [23] The interaction between target and the ligand proposed in this study (Fig 3 to 6) is useful to understanding the potential mechanism to of enzyme and substrate binding in treatment of cancer cell. [24] Hydrogen bond play important role for the structure and function of biological molecules, especially for the enzyme reactions. In the present study, it was found that the active site reside of 3mof template position 220 found least energy, -9.85288 k/mol and formed stronger hydrogen bond interaction with GSH ligand. Active site prediction tools, Hex and Medock showed energy minimization and re-ranking (various algorithms methods) of best poses. The 3mof have been showed to catalyze a number of reaction involved in the process of synthesizing i.e. Glucose Metabolic Process, Oxaloacetate Metabolic Process, Lipid Metabolic Process, etc. [25] In the present study definitely support for any process/activity of a cell or an organism in terms of movement secretion, enzyme, production gene expression and etc. for considered as drug/ligand target. Furthermore, the software tool for homology modeling, simulation and structural based virtual screening and online data base are turn enhancing the rapid development in building the repertoire of the receptor and ligand library.

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