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Comparative Structure Modeling of Glyoxalase II Protein of *Oryza sativa*

Ramandeep Kaur Jhinjer*, Naveen Duhan and Parveen Chhuneja

School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab

*Corresponding author:

ABSTRACT

Keywords

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Glyoxalase I (gly I) and Glyoxalase II (gly II), zinc-binding enzymes of the glyoxalase pathway, carry out catabolism of methylglyoxal (MG). *Gly II* gene cloned from *Oryza sativa* has been widely used in genetic transformation experiments for salinity tolerance. In the present study, we tried to propose a potential model of the *Oryza sativa* gly II protein generated via SWISS-MODEL, CPH models 3.0 Server, I-TASSER, HHPred and MODELLER software. Thirteen different models were constructed using different tools and were further optimized by performing Energy minimization and Loop building techniques. The final model was suggested after analyzing and validating the structure by using Structural Analysis and Verification Server (SAVS). Further functional characterization of the modeled protein was accomplished by tool COFACTOR which involves prediction of functional active site region and binding site region. This model can be used for further analysis for different studies.

Introduction

The glyoxalase system is ubiquitous in nature and consists of two enzymes: glyoxalase I (gly I) and glyoxalase II (gly II) glyoxalase enzymes which are important for detoxification of MG to D-lactate using glutathione (GSH) as cofactor which is formed primarily as a byproduct of carbohydrate and lipid metabolism (Veena *et al.*, 1999). The reactions of glyoxalase system are shown in figure 1.

MG is a mutagenic and cytotoxic compound which arrest growth, react with DNA and protein and increase sister chromatid exchange. Besides detoxification of MG, the glyoxalase system could also play a role in oxidative stress tolerance by recycling reduced glutathione (GSH) that would be

trapped nonenzymatically by MG to form hemithioacetal, thereby maintaining glutathione homeostasis (Singla-Pareek *et al.*, 2003).

With a desire to investigate and analyze the effect of metabolic engineering of the glyoxalase pathway with respect to salinity tolerance, *gly II* gene was isolated from *Oryza sativa* L cv IRBB10 (GenBank accession no. AY054407) cloned and transferred into the model plant, tobacco (Singla-Pareek *et al.*, 2003) and rice (Singla-Pareek *et al.*, 2008).

In contrast to gly I, which is found as a single isozyme, gly II exists as multiple isozymes in many organisms, including yeast, plants, and animals. Gly II activity has been found in

both the cytosol and mitochondria. As a first step in understanding the structure and function of mitochondrial gly II enzymes, a mitochondrial isozyme of gly II from *Arabidopsis thaliana* was cloned, over-expressed, purified, and characterized using metal analyses, EPR and ^1H NMR spectroscopies, and X-ray crystallography (Marasinghe *et al.*, 2005).

Naturally occurring homologous proteins have similar protein structure. Three-dimensional protein structure is evolutionarily more conserved due to sequence conservation. Because protein structures are more conserved than DNA sequences, detectable levels of sequence similarity usually imply significant structural similarity (Marti-Renom *et al.*, 2000).

Homology models of proteins are of great interest for planning and analyzing biological experiments when no experimental three dimensional structures are available. In the present study, we generated *in silico* three dimensional (3D) structure of *Oryza sativa* gly II protein based on the available template structural homologues from Protein Data Bank and the model validated with standard parameters. This study could prove useful in further functional characterization of this important protein.

Materials and Methods

The protein sequence of *Oryza sativa* gly II (NCBI protein accession number AAL14249.1) examined in this study was retrieved from the database NCBI (Singla-Pareek *et al.*, 2003). Structurally homologous subsets of the experimentally determined 3D structures of the *Oryza sativa* gly II protein retrieved from PDB. Protein Sequence (336 amino acids) of *Oryza sativa* gly II was used for alignment with BLASTp by PSI BLAST option. Highest score was obtained with

Chain A, X-Ray Structure of Gene At2g31350 (PDB: 1XM8_A). This protein is made up of 254 amino acids. Gly II from *Arabidopsis thaliana* showed 75% query coverage and 71% maximum identity.

Target-template alignment

Protein Sequence of gly II of *Oryza sativa* (336 amino acids) and *Arabidopsis thaliana* (254 amino acids) was aligned by Multiple Alignment Tool TCOFFEE.

Secondary Structure Prediction

Secondary Structure of gly II protein of *Oryza sativa* was obtained by using I-TASSER online tool (which was used for target protein predicted Model).

Comparative Structure Modeling

Tertiary structure of the "target" protein from its amino acid sequence is build by homology modeling by using four online tools (SWISS-MODEL, CPH models 3.0 Server, I-TASSER, HHPred) and one offline tool (MODELLER). From these five servers, Both I-TASSER and MODELLER each tool generated five possible models of target protein and other each three tools generated one possible model.

Model Refinement Using Energy Function

Swiss-PdbViewer (aka DeepView) was used to check Ramachandran Plot of each 13 possible models of target protein and for Energy Minimization.

Five Models of target proteins which were generated by I-TASSER server showed 11 to 16 amino acids in disallowed region of Ramachandran Plot. These five were not included further in Loop Building experiments to change location of amino

acids from disallowed region to allowed region. Left 3 Models which were generated by online tools were chosen for loop building (Table 1).

Same in case of five Models which were generated by offline tool, loop building experiment was done. Based on minimum energy and Objective function, Model-5 of MODELLER tool was selected from five models because the final energy minimization value of Model-5 was -5623.540 KJ/mol (Table 2).

Model evaluation

After loop building and Energy Minimization, four Models (generated by SWISS-MODEL, CPH models 3.0 Server, HHPred, MODELLER) were evaluated by SAVES (Structural Analysis and Verification Server). SAVS is a server for analyzing protein structures for validity and assessing how correct they are. It utilizes 5 programs for doing this: PROCHECK, WHAT_CHECK, ERRAT, VERIFY_3D, PROVE) (Tables 3 and 4) (Figures 2 and 3).

Table.1 Energy and amino acids (in Ramachandran Plot) in models generated by online tools

Model	Amino acids in Disallowed Region	Energy value before energy minimization	Energy value after energy minimization
CPH	01	-10947.196	-14744.631
Swiss-Model	01	-11308.413	-14049.546
HHPred	01	-2780.819	-15048.808
I-TASSER			
Model 1	12	-1957.130	-
Model 2	15	-3363.378	-
Model 3	16	-2705.362	-
Model 4	11	+68.646	-
Model 5	15	-3301.194	-

Table.2 Energy and amino acids (in Ramachandran Plot) in models generated by offline tool (MODELLER)

Model	Objective Function	Amino acids in Disallowed Region	Energy value before energy minimization	Energy value after energy minimization
Model 1	2048.4500	10	+4073.675	-5278.456
Model 2	2029.8680	12	+4508.503	-4976.345
Model 3	2000.6361	8	+3768.075	-5569.560
Model 4	2024.5565	12	+6605.753	-5146.675
Model 5	1859.8506	8	+5397.916	-5623.540

Table.3 Results of PROCHECK

Results of PROCHECK		Template Model of Arabidopsis	Predicted Models of <i>Oryza sativa</i>			
		1xm8_A	Modeller (Model 5)	HHPred	CPH	Swiss Model
Ramachandran plot (Amino acids in allowed region) (%)		100	100	100	100	100
All Ramachandrans (labelled residues)		1 /252	14/177	7/334	11/252	3/252
Chi1-chi2 plots (labelled residues)		0/155	3/102	0/188	3/150	1/150
Main-chain parameters	Better	5	5	6	6	6
	Worse	1	1	0	0	0
Side-chain parameters	Better	5	5	5	5	5
	Worse	0	0	0	0	0
Residue properties	Max. Deviation	5.3	6.7	8	6.8	5.3
	Bad Contacts	46	0	0	0	0
	Bond Length/ Angle	4.9	6.3	5.1	4.8	3.9
M/c bond lengths: within limits (%)		98.2	100	100	100	100
M/c bond angles: within limits (%)		97.6	94.3	97.1	97.1	97.1
Planar groups: within limits (%)		96.6	60.7	67.3	78.9	67.4

Table.4 Results of WHAT_CHECK, ERRAT and VERIFY_3D Tools

Tools		Template Model of Arabidopsis	Predicted Models of <i>Oryza sativa</i>			
		1xm8_A	Modeller (Model 5)	HHPred	CPH	Swiss Model
What_check	Errors	5	8	7	7	6
	Warnings	18	16	14	20	11
Verify_3D (%)		98.82	75	75.07	100	100
Errat (Overall quality factor)		92.683	69.591	95.181	65.447	86.885

Fig.1 The reaction catalysed by glyoxalase I and glyoxalase II (Veena *et al.*, 1999)

Fig.2 Ramachandran Plots generated by PROCHECK

(A) Protein Model of MODELLER

(B) Protein Model of Swiss Model

Fig.3 Results of ERRAT of

(A) Protein Model of MODELLER

(B) Protein Model of Swiss Model

Fig.4 (A) 3D structures of gly II of *Arabidopsis thaliana*; (B) Predicted 3-D structure of gly II of *Oryza sativa* (from Swiss Model) created by Rasmol

Fig.5 Predicted (A) Active sites (B) Binding sites of *Oryza sativa* gly II

Fig.6 Comparison structure of template and target proteins (Green: Template, Purple: Swiss Model of Target)

Functional characterization of *Oryza sativa* gly II protien

Functional characterization of *Oryza sativa* gly II protein was done by COFACTOR (<http://zhanglab.ccmb.med.umich.edu/COFACTOR/>) which is a structure-based method for biological function annotation of protein molecule. To use COFACTOR, there is need of a 3D-structural model of the protein of interest. Functional insights, including ligand-binding site, gene-ontology terms, and enzyme classification, were then derived from the best functional homology template.

Results and Discussion

Comparative modeling to build 3-D structure of the gly II protein (*Oryza sativa*) was made based on the experimentally solved structural homologues. The amino acid sequences of gly II protein (*Oryza sativa*) were submitted to five servers and atomic coordinates for the proteins were generated. The hypothetical protein models created were stored as PDB output file. The hypothetical proteins were visualized and computed by Swiss PDB Viewer, Rasmol and Chimera. After model evaluation step, model generated by Swiss-Model Tool was selected as best model from all four possible models. The 3-D structure of the protein was represented by cartoon

display and colored based on the secondary structure (Figure 4 and 6). Functional characterization of putative protein structure of *Oryza sativa* gly II by COFACTOR tool revealed the predictive active sites and binding sites (Figure 5). Sequence search in Pfam online tool indicated the metallo-beta-lactamase protein fold that confirmed the target protein sequence of *Oryza sativa* as gly II protein.

References

- Marasinghe, G.P.K., Sander, I.M., Bennett, B., Periyannan, G., Yang, K.W., Makaroff, C.A. and Crowder, M.W. 2005. Structural Studies on a Mitochondrial Glyoxalase II. *J. Biol. Chem.*, 280(49): 40668–40675.
- Marti-Renom, M.A., Stuart, A.C., Fiser, A., Sanchez, R., Melo, F., Sali, A. 2000. Comparative protein structure modeling of genes and genomes. *Annu Rev Biophys. Biomol. Struct.*, 29: 291–325.
- Singla-Pareek, S.L., Reddy, M.K. and Sopory, S.K. 2003. Genetic engineering of the glyoxalase pathway in tobacco leads to enhanced salinity tolerance. *PNAS*, 100(25): 14672–14677.
- Singla-Pareek, S.L., Yadav, S.K., Pareek, A., Reddy, M.K. and Sopory, S.K. 2008. Enhancing salt tolerance in a crop plant

- by overexpression of glyoxalase II. *Transgenic Res.*, 17: 171–180.
- Veena, Reddy, V.S. and Sopory, S.K. 1999. Glyoxalase I from *Brassica juncea*: molecular cloning, regulation and its over-expression confer tolerance in transgenic tobacco under stress. *The Plant J.*, 17(4): 385–395.

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