**Interaction Studies of Heat Shock Responsive Protein HSA32 and Heat Shock Protein HSP101:
An In Silico Approach**

Mahmood-ur-Rahman^{1*}, Waseem Akhtar Shamshari², Sadia Ali¹, Khadim Hussain¹, Muhammad Qasim¹,
Shazia Anwar Bukhari²

¹Plant Research Group, Department of Bioinformatics and Biotechnology, GC University, Allama Iqbal Road, Faisalabad-38000, Pakistan.

²Department of Applied Chemistry and Biochemistry, GC University, Allama Iqbal Road, Faisalabad-38000, Pakistan.

*Corresponding Author E-mail: mahmood1233@yahoo.com; mahmoodansari@gcuf.edu.pk
Phone: +92-301-3967919

Research Article

ABSTRACT

Heat shock responsive protein HSA32 is a good candidate responsible for heat stress tolerance in crop plants. It interacts with other proteins in a signaling pathway which results its regulation of gene expression in response to heat shock. HSP101 is such protein which in combination with HSA32 is essential to thermotolerance in crop plants. In this study, we constructed 3-D structures of the two proteins. Hypothetical point mutations were also induced in HSA32 protein at amino acid position 27, 91, 159 and 220 and their 3-D models were developed. Then, protein – protein interaction was studied between two proteins. Our results showed that HSA32 and HSP101 proteins interact with each other in the active binding sites. It was also found that mutants HSA32 E<Q at amino acid position 91 and HSA32 G<A at the position 159 were interacting with HSP101 protein while HSA32 E<G at position 27 and HSA32 K<R showed no interaction with HSP101 protein. So, it is concluded that HSA32 interacts with HSP101 protein and both in combination are responsible for the acquired thermotolerance in crop plants. It is also concluded that point mutations in the protein active binding sites results in loss of function and there is no positive interaction between two proteins.

Keywords: HSA32, protein – protein interaction, HSP101, thermotolerance, heat shock proteins

INTRODUCTION

When the plant cells are exposed to high temperature, some self defense mechanisms are activated called as heat shock response (HSR) [1]. There are lots of genes involved in this mechanism. *Hsa32* is a good candidate for this purpose. It is observed that *Hsa32* was up-regulated by heat shock treatment in plants [2]. It shows its important role in HSR during plant evolution. *Hsa32* and its homologs are only found in land plants and certain microorganisms. Plant HSA32 shares approximately 35% sequence similarity with (2R)-phospho- 3-sulfolactate synthase. This enzyme catalyzes the coenzyme M biosynthesis in *Methanococcus jannaschii*. It is thought that the *Hsa32* has been originated in plants by horizontal gene transfer [3]. The ubiquitous presence of the *Hsa32* gene in plants indicates that it may function as a

member of some self-defense mechanism of plants against high temperature.

HSP101 is a member of heat shock protein family. It acts as molecular chaperone [4, 5, 6]. HSP104, the yeast (*Saccharomyces cerevisiae*) counterpart of HSP101, was shown to resolubilize heat-denatured proteins from insoluble aggregates [7, 8] and responsible for thermotolerance [9]. A yeast mutant lacking HSP104 could not acquire thermotolerance [10, 11] which shows its role in the defense mechanism of plants. In addition to its role in thermotolerance, HSP101 has also been suggested to increase translation activity [12, 13].

Keeping in view the roles of two proteins, it is hypothesized that they interacts and complement with each other and activates self-defense mechanism in the plants when they are exposed

to high temperature. So, this project was designed to study the process of interaction between HSA32 and HSP101 proteins which may help us to understand the role of two proteins in thermotolerance mechanism.

MATERIALS AND METHODS

Retrieval of Sequences and Induction of Mutations

Nucleic acid sequences of *has32* (Acc. No. NM_118250) and *hsp101* (Acc. No.

NM_106091.3) genes were retrieved from NCBI database (<http://www.ncbi.nlm.nih.gov/nucleotide>). Protein sequences of HSA32 (Acc. No. NP_567623) and HSP101 (Acc. No. NP_565083) were retrieved from protein data bank of NCBI database (<http://www.ncbi.nlm.nih.gov/protein/>).

Hypothetical point mutations were induced to the *has32* gene which encodes an altered protein at amino acid position 27, 91, 159 and 220 (Table-1).

Table-1: Heat stress associated 32 (*has32*) gene and its mutations

S. No.	Gene Name	Accession No.	Position of Mutation	Codon Modified		Amino acid Modified	
				Original	Modified	Original	Modified
1.	Heat Stress Associated 32 (<i>has32</i>)	NM_118250	27 (E<G)	GAA	GGA	Glutamic acid (E)	Glycine (G)
			91 (E<Q)	GAA	CAA	Glutamic acid (E)	Glutamine (Q)
			159 (G<A)	GGC	GCC	Glycine (G)	Alanine (A)
			220 (K<R)	AAA	AGA	Lysine (K)	Arginine (R)

Protein Modeling and Active Site Determination

3D structures of HSA32 and HSP101 proteins were modeled by using online server SWISS-MODEL Workspace (<http://swissmodel.expasy.org/workspace/>) and were evaluated by Ramachandran Plot. The active sites of proteins were not reported previously, so the active sites for protein binding were determined by online server Pocket Finder (<http://www.modelling.leeds.ac.uk/pocketfinder/>).

Protein – protein Interaction

HADDOCK (High Ambiguity Driven protein-protein Docking) (<http://haddock.science.uu.nl/services/HADDOCK/haddock.php>) was used to study protein-protein interactions. HADDOCK is an information-driven flexible docking approach for the modeling of bimolecular complexes. The results derived from HADDOCK were further visualized and evaluate by using molecular visualization system on an open source foundation, PyMOL (<http://www.pymol.org/>). These interactions were viewed and compared with control and discussed.

Figure-2: Protein structures (3-D models) of HSP101 protein. A-B) 3-D model of HSP101-A and its evaluation by Ramachandran plot and C-D) 3-D model of HSP101-B and its evaluation by Ramachandran plot.

RESULTS

Development of HSA32 and HSP101 Protein Models

Protein model of HSA32 was developed and evaluated by Ramachandran plot (Figure-1A-B). There were two models developed for HSP101 protein. Model A starting was developed from

the amino acids 160-353 while model B comprised of amino acids 568-860 (Figure-2). Models for HSA32 mutants were also developed. All the models were evaluated by Ramachandran plot and found that more than 90% residues clustered in allowed region (Figure-1C-J)

Figure-1: Protein structures (3-D models) of HSA32 protein and its mutants. A-B) 3-D model of HAS32 protein and its evaluation by Ramachandran plot, C-D) 3-D model of HAS32 E<G and its evaluation by Ramachandran plot, E-F) 3-D model of HAS32 E<Q and its evaluation by Ramachandran plot, G-H) 3-D model of HAS32 G<A and its evaluation by Ramachandran plot and I-J) 3-D model of HAS32 K<R and its evaluation by Ramachandran plot.

Determination of Active Sites

According to the results of pocket finder based on Ligsite algorithm [14], HSP101-A has binding site residues LEU171, GLN174, ALA175, LEU178, ASP179, PRO180, VAL181, GLU218, GLY219 and GLN222 while binding sites in HSP101-B were MET781, VSL784, SLS785, LEU795, VSL797, SLS801, LEU802, ILE805, MET823, VSL827, VSL828 and

ILE848. The active binding sites for HSA32 and its mutants were also found with the same procedure and the data is presented in the Table-2.

Protein-Protein Interaction

HSP101-A and HSP101-B were first docked with HSA32 by providing specific binding residues which show site specific interactions. Then HSP101-A was docked with mutants of

HSA32 which showed proper interactions with mutant HSA32 E<Q and HSA32 G<A. There were some similar residues of HSA32, mutant HSA32 E<Q and HSA32 G<A interacting with HSP101-A. Similarly there were also some residues of HSA32, mutant HSA32 E<Q and HSA32 G<A interacting with HSP101-B. No interaction was found between either of HSP101-A or HSP101-B and HSA32 E<G and HSA32

K<R mutants (Table-2). The interacting residues of both HSA32 protein and HSA32 E<Q which showed interactions with HSP101-A were Lys 138, Arg 143, Lys 229, Asp 53, Glu 228, Asn 252, His 78, Gln 50, Arg 22 while the residues of HSA32 and HSA32 E<Q which showed similar interactions with HSP101-B were Lys 8, Glu 264, Arg 22, Gln 259.

Table-2: Interaction of HSA32 and HSP101 proteins and their binding residues

S. No.	Clusters	Z-score	HADDOCK score	Electrostatic energy	Residues showing interactions
1.	HSP101-A_HSA32	-1.8	-72.2 ± 6.7	-830.0 ± 55.6	GLN162 - ASN139, THR166 - ASN139, LYS 65 - LYS 138, THR 166 - LYS 138, LYS 165 - ASP144, ASN 160 - LYS109, ASP 170 - ARG143, GLU 173 - ARG143, GLU 173 - THR115, GLN 174 - LYS229, GLN 174 - ASP 53, ARG 352 - GLU228, LYS177 - ASP53, ARG352 - ASN252, ARG352 - VAL 251, ARG237 - HIS78, ARG237 - GLY 81, VAL 225 - GLN 50, ASP 179 - ARG 22, LEU351 - ARG22, ILE350 - ARG22
2.	HSP101-B_HSA32	-2.0	-65.2 ± 10.7	-728.2 ± 128.1	ASN 628 -ARG 16, GLU 679 -LYS 8, ARG 817 - ASP 15, ARG 820 - PRO 20, ARG 821 - GLU 264, ASP811 - GLN 50, ASP 783 - ARG20, GLU 830 - LYS229, ASP854 - TYR 83, ARG 817 - GLU 12, ARG 632 - ASP 15, ASP-634 - SER 9, ASP 783 - ARG 22, SER 636 - LYS 8, ARC 827 - GLU193, GLU 179 - GLN 259, SER 836 - LYS 8
3.	HSP101-A_HSA32 E<Q	-1.9	-70.3 ± 7.1	-822.0 ± 60.5	GLU 186 - LYS 273, ARG 184 - ASP 262, VAL 181 - ASP-262, ARG 189 - ASP 11, ARG 189 - ASP 236, LYS 226 - ARG 29, LYS 226 - ASP 256, SER 349 - TRP 7, ARG 352 - SER 47, ASP 179 - SER 47, LYS 277 - GLU 43, LYS 277 - SER 37
4.	HSP101-B_HSA32 E<Q	-2.1	-68.2 ± 9.8	-738.2 ± 118.2	HIS 568 - HIS 275, ASP 771 -LYS 8, Lys 775 - GLU 11, LYS 782 - ASP 262, ASP799 - ARG 6, ARG 778 - GLU 264, ARG 778 - MET261, ALA 785 - GLN 259, VAL 793 - GLN259, ARG 858 - GLU 46, TYR 847 - GLU 43, GLY 792 - SER 258, GLU 842 - TYR 33
5.	HSP101-A_HSA32 G<A	-1.8	73.5 +/- 6.8	-834.0 +/- 56.7	LEU 351 - ARG 22, ILE 350 - ARG 22, ARG 352 - GLU 228, ASP 179 - ARG22, ASP 179 - TYR 23, ARG 352 - ASN 252, LYS 226 - GLN 50, ASP235 - HIS 78, LYS 165 - ASP 114, LYS 165 - GLY 141, LYS 165 - LYS 138, THR 166 - LYS 138, GLN 174 - ARG 143, ASP 170 - ARG 143, LYS 177 - LYS 229, ARG 237 - ASP 114, LYS 177 - ASP 53, LYS 177 - TYR 83
	HSP101-B_HSA32 G<A	-2.1	-70.4 ± 8.1	-749.3 ± 114.8	LYS 775 - GLY 140, LYS 775 - GLY 141, ASP799 - ARG 143, VAL 797 - ARG 143, ASP799 - THR 115, ARG 774 - ASP 114, ASP 803 - HIS 78, TYR 804 - VAL 52, GLY851 - ASP 53, ALA 850 - ASP 53, VAL 859 - ARG 22, GLU 839 - ARG 22, VAL 797 - THR 198, ARG 858 - GLU 228, ARG 858 - THR 230

Note: Residues in Bold letters are of Protein A and Protein B

DISCUSSION

In this research article, we reported molecular and computational analysis of HSA32 and HSP101 proteins and protein – protein interactions between them. Gene expression studies of *Hsa32* and *hsp101* under heat stress indicate its importance in thermotolerance of plants. To understand the mechanism and function of certain gene, a well-documented protocol is knocking out the gene under study and studies its phenotype. However, is difficult because of redundancy of the gene or signaling networks [15] (Cutler and McCourt, 2005) and requires extensive experimentation. It also requires longer time to study all aspects. So, it is desired to adopt an in silico approach to find the role of certain gene in a particular mechanism.

In this study, we showed that HSA32 interacts with HSP101 protein and is greatly affected by mutations. The mechanism by which HSA32 interacts with HSP101 is not clear; however, our results indicated that these two proteins have direct interaction. Wu et al. [2] studied the interaction between HSA32 and HSP101 in the laboratory. They could not find a protein corresponding in size to HSA32 in an immunoprecipitation of HSP101 [2]. Our results do not agree with them due to unknown reasons. By controlling HSP101 degradation, HSA32 prolongs the effect of HSR. It allows plants to cope with rapid increase in the temperature [16]. This hypothesis is in line with our studies and shows positive correlation between the two proteins. It is further suggested that a primary role of HSP101 is in emergency heat tolerance [16]. It is also suggested that HSA32 complements HSP101 when plants are exposed to high temperature and activates thermotolerance mechanism. Heat induced HSP101 decayed differentially in indica and japonica rice [11]. It suggests a role of regulating HSP101 decay in the adaptation to changing climates. Very recently, using rice Tos17 retrotransposon insertion lines, it is observed that disruption of HSA32 also led to faster decay of HSP101 in Arabidopsis [2]. Future investigations on the structure-function association of HSA32 and HSP101 in plant species will provide us

more insight into the high temperature tolerance mechanism.

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