

MOLECULAR DOCKING OF HSPs (HEAT SHOCK PROTEINS)  
FROM RICE SEED

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## ABSTRACT

The expression of heat shock proteins (HSPs) is an essential part of the heat shock response in plants where HSPs act as chaperones that help in protein folding and unfolding mechanism. This project focuses on determining the docking mechanism and conformation stability of HSPs from rice seed at several simulated temperatures. HSP20 was selected from previously isolated rice seed proteins and the sequence was used for homology modelling. The suitable model of this sequence was then selected to undergo molecular dynamics simulation and docking procedure with the targeted proteins. The simulated environmental temperature was set as 37°C and 100°C during the simulation process. Model for the selected HSP20 protein was generated successfully via I-Tasser server. The results of 50ns simulation at 310K and 373K for selected model were plotted in the graphs of RMSD, RMSF, hydrogen bonding number and radius of gyration. The RMSD result showed that HSP20 model was more stable at simulated temperature of 37°C as compared to 100°C. Whereas for the RMSF graph visualization, two significant loops of this model were found in the same position of its 3D structure as corresponded in both 37°C and 100°C results. For the results of hydrogen bonding number and radius of gyration, the mean numbers were around 102 and 1.6 respectively for 37°C and 100°C simulations. These findings indicated the responsible stability and flexibility of the model's 3D structure in terms of its secondary structure, folding pattern and loops location. Molecular docking of this HSP20 model with the selected proteins: TPR and SGT1 was carried out successfully with ZDock server. The docked structures generated were used to understand the docking mechanism and protein-protein interactions between these proteins. Further study of these protein models is required for understanding on the roles of binding conformation between them as well as possible protein-protein interaction of HSP20 with other co-chaperones.

## ABSTRAK

Ekspresi heat shock proteins (HSPs) atau protein tahan haba adalah suatu tindak balas tahan haba oleh tumbuh-tumbuhan. HSPs berperanan sebagai ‘chaperones’ dengan membantu dalam mekanisme penglipatan protein. Kajian ini menumpu pada mekanisme dok dan kestabilan struktur HSPs daripada beras padi dalam beberapa suhu simulasi. HSP20 dipilih daripada kandungan protein beras padi terpendek terdahulu dan digunakan untuk pemodelan homologi. Model yang sesuai seterusnya dipilih demi penjalanan simulasi dinamik molekul. Suhu simulasi ditetapkan pada 37°C dan 100°C sepanjang proses simulasi. Model protein HSP20 telah berjaya dihasilkan dengan server I-Tasser. Hasil simulasi bagi 50ns pada suhu 310K dan 373K telah diplotkan dalam graf-graf RMSD, RMSF, jumlah ikatan hidrogen serta radius gyration. Graf RMSD menunjukkan bahawa model HSP20 adalah lebih stabil pada suhu simulasi 37°C berbanding dengan 100°C. Manakala dalam graf RMSF, didapati kedua-dua gelung dalam model ini berada pada kedudukan yang sama seperti 3D strukturnya sebagaimana dalam kehasilan simulasi pada 37°C and 100°C. Dalam graf-graf jumlah ikatan hidrogen dan radius gyration, nombor mean adalah kira-kira 102 dan 1.6 masing-masing bagi kedua-dua simulasi pada 37°C dan 100°C. Penemuan ini juga bersesuaian dengan keputusan struktur 3D bagi model HSP20 dari segi struktur sekunder, bentuk lipatan dan lokasi gelung yang berkenaan. Proses dok molekul bagi model HSP20 dengan protein TPR dan SGT1 telah berjaya dijalankan dalam server ZDock. Struktur-struktur hasil proses dok telah digunakan untuk memahami mekanisme dok serta interaksi protein-protein antara mereka. Kajian lanjut bagi model-model ini adalah diperlukan bagi pemahaman berhubung peranan ikatan dalam konformasi mereka serta kemungkinan interaksi protein-protein antara HSP20 dengan protein ‘co-chaperone’ yang lain.

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**LIST OF SYMBOLS**

|                    |   |                |
|--------------------|---|----------------|
| $\alpha$           | - | Alpha          |
| $\beta$            | - | Beta           |
| $^{\circ}\text{C}$ | - | Degree Celcius |
| K                  | - | Kelvin         |
| kDa                | - | Kilo Dalton    |
| nm                 | - | Nanometer      |
| ns                 | - | Nanosecond     |
| %                  | - | Percentage     |

**LIST OF ABBREVIATIONS**

|               |   |                                     |
|---------------|---|-------------------------------------|
| CHORD         | - | Cysteine and histidine-rich domain  |
| <i>et al.</i> | - | And friends                         |
| HSP           | - | Heat Shock Protein                  |
| kDa           | - | Kilo Dalton                         |
| Mb            | - | Megabases                           |
| MD            | - | Molecular Dynamics                  |
| NMR           | - | Nuclear Magnetic Resonance          |
| PDB           | - | Protein Data Bank                   |
| Rg            | - | Radius of gyration                  |
| RMSD          | - | Root Mean Square Deviation          |
| RMSF          | - | Root Mean Square Fluctuation        |
| SGT1          | - | Suppressor of G2 allele of SKP1     |
| SKP1          | - | S-Phase Kinase Associated Protein 1 |
| TPR           | - | Tetratricopeptide Repeat            |

## CHAPTER 1

### INTRODUCTION

#### 1.1 Background Information

One common environmental variable encountered by organisms is the surrounding temperature that influences homeostasis maintenance. High temperature will cause negative impacts that interrupt cellular homeostasis and physiology at various growth stages of plants. A complex signaling network is therefore constructed by plants to minimize the effects of heat stress. This involves rapid induction of genes with the expression of heat shock proteins (HSPs), which acts as an essential part of the heat shock response (Zhang *et al.*, 2016). In general, HSPs are categorized in terms of their molecular weight and have been grouped in families including HSP90, HSP70, HSP60, HSP40, and small HSPs (sHSPs). Basically, HSPs act as chaperones which help in protein folding and unfolding mechanism. They also served as stress-response factors that have vital role in a range of cell processes, such as apoptosis, cell cycle and division, development as well as differentiation (Martín-Folgar *et al.*, 2015).

Rice (*Oryza sativa* L.) can be referred as one of the critical crops in eastern Asia. Numerous rice cultivars and wild species of rice have been widely grown for identification

of their genetic and molecular makeup. According to the study of Bernier and co-workers, rice ecosystems can be divided into four major categories: deep-water, irrigated, rainfed lowland and rainfed upland (Bernier *et al.*, 2008). On the other hand, rice is also considered as model plant in monocot category due to its small size genome which consists of about 430 million base pairs and around 30,000 genes responsible for proteins production (Komatsu *et al.*, 2003). When it comes to proteomics in rice, it basically involved the study of proteomes in various parts of rice. These proteins are resulted from specific gene expression under various growth and developmental conditions, as well as under different abiotic and biotic stresses. Kim and co-workers mentioned about technological development and improvement in both gel-based and gel-free approaches, especially MS analysis and bioinformatics resources in plant proteomics have contributed greatly to the global advance of rice proteomics analyses (Kim *et al.*, 2014).

## 1.2 Problem Statement

HSPs expression can be referred as the strategy of plants to cope with the several biotic and abiotic stresses as well as environmental changes due to their sessile properties. Study regarding the mechanism of HSPs in plants is particularly important in understanding of their functions and discover more of their potential roles. Although currently there are some studies on HSPs from rice plants, but molecular docking of HSPs sequences isolated from rice seed is still not available. Moreover, there is also a lack of research on binding mechanism of HSP20 with co-chaperones or other proteins, which is crucial for HSPs role in plant immunity. Thus, a study on comparison of selected HSP20 conformation under different simulated temperature would provide preliminary information before proceeding to the determination of HSP20 stability and its docking mechanism followed by the prediction on protein-protein interactions of HSP20 with the chosen proteins.



### **1.3 Objectives**

1. To determine the stability of HSP20 at 37°C and 100°C
2. To determine the docking mechanism of HSP20
3. To predict the protein-protein interaction of HSP20 with coat protein, TPR (Tetratricopeptide Repeat) and SGT1 (Suppressor of G2 allele of SKP1)

### **1.4 Significance of Work**

This project focuses on determining the docking mechanism and conformation stability of HSPs from rice seed at several simulated temperatures. Current studies on HSPs are mainly based on animal sources rather than plants for eukaryotes and study regarding rice HSPs is very few. There is still absent of work related to molecular docking of HSPs from rice seed. Hence, this study could provide information for structure stability of selected HSPs at different simulated temperatures as well as possible interaction between HSPs and the chosen ligands.

### **1.5 Scope of Work**

HSP20 was selected from previously isolated rice seed proteins (Lee, 2015) and these HSP20 sequences were used for homology modelling. The suitable model of the chosen HSP20 sequence was proceeded with molecular dynamics simulation. The simulated environmental temperatures were set as 37°C and 100°C during the 50ns MD

run. The stability and flexibility of the selected HSP20 model can therefore be identified. Molecular docking of HSP20 model with the targeted TPR and SGT1 proteins was also be carried out as for the prediction of protein-protein interactions between these docked structures could be achieved successfully.

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