COLD ADAPTATIONS STUDY OF GLYCOSYL HYDROLASE ENZYMES VIA COMPUTATIONAL METHODS

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“Dedicated to my beloved husband, my son, my parents and parents-in-law”

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ABSTRACT

Psychrophiles are cold loving organisms that have adapted to live in permanently cold environments. These microorganisms synthesize psychrophilic enzymes with high catalytic efficiencies at cold temperaturesranging from -20°C to +10°C. This research intends to perform an *in silico* analysis of the cold adaptation of *Glycosyl hydrolase* enzymes isolated from psychrophilic yeast *Glaciozyma antarctica*. Two enzyme were selected; *β-mannanase* (PMAN) and *β-glucanase* (PLAM) from two different *glycosyl hydrolase* families with different domains. A 3D model was predicted for both genes using a fold recognition method. The proteins were comparatively studied against their mesophilic, thermophilic, and hyperthermophilic counterparts. The study of these enzymes illustrates that they mostly use similar strategies for cold adaptation.The structure of PLAM and PMAN consist of longer loops in three different positions. Their structure also has several amino acids substitution including increased number of alanine, glycine, and polar residues and decreased number of proline, arginine, and hydrophobic residues. The PLAM and PMAN structure showed longer motions around the entrance region to active site. A lower number of salt bridges and H-bonds have been observed in the PLAM and PMAN structure. PLAM consists of 5 salt bridges while its homologous proteins have 9, 7, and 18 salt bridges, respectively. Also, the number of H-bonds per residue is 0.54 where it is 0.62, 0.63, and 0.70 for its homologous counterparts. Furthermore, PMAN includes 5 salt bridges in its structure while its homologous counterparts have 10, 14, and 21 salt bridges, respectively. The number of H-bonds per residue for PMAN is 0.62 while it is 0.71, 0.73 and 0.78 for its homologous counterparts. The PLAM structure has 41% of secondary structure, while its homologous counterparts have 54%, 58%, and 60% of secondary structure. Also, this percentage is 47% for PMAN, and 48%, 50%, and 53% for its homologous proteins. Additionally, they also use different strategies related to the role of salt bridges in their structure. The PLAM structure contains alternative salt bridges connecting inner and outer leaflets, while the PMAN structure includes weakly linked salt bridges between residues located on a loop instead of β-sheet. In conclusion, *in silico* analysis of two psychrophilic proteins revealed novel characteristics of these cold adapted enzymes. The analysis showed the adopted strategies by these two proteins in contributing to the general and local flexibility of their structure and increase capability of the enzymes to be active at cold temperatures. The presented findings in this research will assist future attempts in the rational design of enzymes with enhanced enzymatic capabilities.

ABSTRAK

Organisma psikrofilik adalah organisma yang telah menyesuaikan diri untuk hidup dalam persekitaran yang sejuk kekal. Mikroorganisma-mikroorganisma ini mensintesis enzim psikrofilik dengan tujuan untuk mengekalkan kecekapan pemangkin pada suhu sejuk antara -20 ° C hingga + 10 ° C. Kajian ini bertujuan untuk melakukan analisis komputeran adaptasi suhu sejuk enzim *glikosil hidrolase* yang telah diasingkan daripada yis psikrofilik *Glaciozyma antarctica*. Dua enzim yang dipencil telah dipilih; *β-mannanase* (PMAN) dan *β-glukanase* (PLAM) daripada dua keluarga enzim *glikosil hidrolase* yang berbeza. Model 3D telah diramalkan untuk kedua-dua gen menggunakan kaedah "pengecaman lipatan". Protein dikaji secara perbandingan terhadap enzim mesofilik, termofilik, dan hipertermofilik. Kajian enzim ini menggambarkan bahawa kebanyakan enzim menggunakan strategi yang sama untuk mengadaptasi kepada keadaan sejuk. Struktur PLAM dan PMAN terdiri daripada gelungan-gelungan pada tiga kedudukan yang berbeza. Struktur mereka juga mempunyai beberapa perubahan asid amino seperti jumlah peningkatan alanina, glisin, jujuk amino polar dan beberapa prolin, arginina, dan jujuk amino hidrofobik yang dikurangkan jumlahnya. Struktur PLAM dan struktur PMAN menunjukkan pergerakkan lebih dinamik di sekitar kawasan pintu masuk ke tapak aktif enzim. Beberapa ciri yang menunjukkan penurunan dalam struktur PLAM dan PMAN adalah jambatan garam dan rangkaian H. PLAM mempunyai 5 jambatan garam manakala homolog mempunyai 9, 7, dan 18 jambatan garam. Bilangan rangkai H untuk setiap jujuk asid amino adalah 0.54 berbanding dengan 0.62, 0.63, dan 0.70 untuk protein homolog. Tambahan pula, PMAN mempunyai hanya 5 jambatan garam dalam struktur berbanding dengan protein homolog yang mempunyai 10, 14, dan 21 jambatan garam. Bilangan rangkai H untuk setiap jujuk asid amino untuk PMAN adalah 0.62 berbanding dengan 0.71, 0.73 dan 0.78 protein homolognya. Struktur PLAM mempunyai 41% daripada struktur sekunder, manakala rakan-rakan homolog yang mempunyai 54%, 58%, dan 60% daripada struktur sekunder. Peratusan ini adalah 47% untuk PMAN, dan 48%, 50%, dan 53% berbanding protein homolog. Selain itu, mereka juga menggunakan strategi yang berbeza untuk menggunapakai jambatan garam dalam struktur mereka. Struktur PLAM mengandungi jambatan garam alternatif menyambung bahagian dalaman dan luaran, manakala struktur PMAN mempunyai jambatan garam yang lemah untuk mengaitkan di antara asid amino yang dua terletak pada gelungan antara lembaran β. Kesimpulannya, analisis komputeran dua protein psikrofilik menunjukkan beberapa ciri-ciri unik yang membolehkan enzim ini berfungsi di dalam suhu sejuk kekal. Analisa ini menunjukkan strategi yang diguna pakai oleh kedua-dua protein dalam menyumbang kepada fleksibiliti secara umum dan khusus terhadap keupayaan struktur dan pengekalan keupayaan mereka menjadi enzim aktif pada suhu yang sejuk. Hasil kajian ini akan membantu dalam memperolehi enzim dengan aktiviti keupayaan tinggi melalui rekabentuk rasional enzim.

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3D	-	Three-Dimensional
AFP	-	Anti-Freeze Protein
DNA	-	Deoxy-ribo Nucleic Acid
GANDB	-	Glaicozyma Antarctica Genome DataBase
GH	-	glycoside hydrolase
HB	-	hydrogen bonds
MD	-	Molecular Dynamic
PDB	-	Protein Data Bank
PCA	-	Principal component analysis
PLAM	-	Psychrophilic Laminarinase
PMAN	-	Psychrophilic Mannanase
RMSD	-	Root Mean Square Deviation
SASA	-	Solvent accessible surface area
SB	-	Salt bridge
TM-score	-	Template Modeling score

CHAPTER 1

INTRODUCTION

1.1 Overview

The wide spectrum of different environments presented by earth's biosphere require a variety of adaptive strategies to be live by organisms. Temperature is the key factor that affects the biochemical adaptation of living organisms to their environment. Organisms inhabiting extreme temperatures have been of particular interest because the isolated proteins from these organisms can remain stable and function at these environments. These proteins are often desirable for industrial processes and engineering proteins from organisms living at moderate temperatures. They also provide a unique opportunity for researchers to study relationships between their structural characteristics and biological functions.

The majority (>80%) of the Earth’s biosphere is permanently exposed to temperatures below 5 °C (Margesin and Miteva, 2011). Psychrophiles are cold loving microorganisms that have adapted to live in permanently cold environments that are close to the freezing point of water. These microorganisms synthesize psychrophilic enzymes with high catalytic efficiencies at cold temperatures. This adaptation requires an adjustment in various cellular components, including the membrane, protein synthesis machinery, energy-generating systems, and other physicochemical characteristics. Enzymes from psychrophiles are supposed to be structurally more flexible than their mesophilic and thermophilic counterparts. This structural flexibility improves the ability of the protein to undergo conformational changes during catalysis and creates an enhanced catalytic efficiency at low temperature with

an inherent decrease in the chemical reaction rates. This establishes the proper plasticity around the active site that is important for the thermolability of enzymes to obtain high catalytic efficiencies at low temperatures (Margesin and Miteva, 2011). These specific characteristics of psychrophilic enzymes provide potential industrial applications in biotechnology and related fields.

Psychrophiles can be found in a large range of microorganisms including *Bacteria*, *Archaea*, and *Eukarya*. They are mostly represented by bacteria (Gounot, 1991; Russell *et al*., 1998), archaea (Siddiqui and Cavicchioli, 2006), algae (Morgan-Kiss *et al*., 2006), yeast (Buzzini *et al*., 2012), plants and animals (Margesin *et al*., 2007; Doucet *et al*., 2009), whereas the biggest psychrophiles are the polar fish thriving beneath the icepack (Eastman, 1993; Prisco *et al*., 1998; Giordano *et al*., 2012). Accordingly, among extremophiles, psychrophiles are the most widely found microorganisms in terms of diversity, biomass, and distribution.

The cold-adapted enzymes have a high biotechnological value due to their high thermolability at raised temperatures, their activity in organic solvents, and their high k_{cat} at low temperatures (Roman *et al*., 2012). The enzymes are more productive than their mesophilic or thermophilic counterparts at low temperature, and thereby, the production processes can be economically done by efficiently saving energy. Therefore, psychrophilic enzymes are widely used in industrial applications such as household molecular biology, detergents, and baking.

1.2 Challenges in characterization of psychrophiles

Psychrophiles synthesize cold-loving enzymes permanently at near-zero temperatures to preserve their cell cycle. The activity of psychrophilic enzymes is mostly optimized at the expense of substrate affinity decreasing the free energy barrier of the transition state. Additionally, the moderate reduction of the catalytic activity at cold environment is ensured by a weak temperature dependence of these enzymes (Struvay and Feller, 2012). Furthermore, activity of enzymes at cold temperature is optimized by destabilization of the whole molecule or the structures

carrying the active site. As a result, the number and strength of all types of weak interactions are decreased, and therefore, dynamics of active site residues in the cold temperature are improved (Struvay and Feller, 2012).

Recently, significant progresses have been obtained to illustrate cold adaptation of enzymes to extreme temperatures. However, there are several questions remain to be answered regarding the structural and functional properties of these psychrophilic macromolecules. The existing challenges include folding reactions at low temperature, kinetic parameters of cold-active enzymes, global and local flexibility of cold-adapted enzymes, macromolecular dynamics, and extreme environmental temperature. Biologists are highly interested to refine their knowledge of the strategies adopted by psychrophilic proteins to be active at cold environment using different related sciences including biochemistry, biophysics, microbiology, and enzymology.

1.3 Problem Statement

Nowadays, the need of enzymes with the capacity to perform their catalysis at low temperature is rapidly increasing. This could be due to their potential environmental application and also their usefulness in industrial processes. Psychrophiles have several remarkable biotechnological potential, which attract researchers to utilize them in several biotechnological applications. Understanding the molecular characteristics and behaviors of these enzymes has an enormous importance to efficiently develop their application in different industries.

Glaciozyma antarctica is a pyschrophilic yeast living at cold, marine, and Antarctic regions. The optimum growth temperature of *G. antarctica* strain PI12 is 12°C (D'Amico *et al.*, 2003) where it can grow up to 18°C. Turchetti *et al*. (Turchetti *et al*., 2011) proposed a new classification of the yeast from *L. antarcticum* to *G. Antarctica* in 2011. Several cold-active proteins have been isolated from this yeast including chitinase (Ramli *et al*., 2012), α-amylase (Ramli *et al*., 2013).

Cold-adapted and heat labile mannanases have been reported from several psychrophilic bacteria, fungi and plants. However, there is no report on a cold-adapted mannanase from psychrophilic or psychrotolerant yeast. Interest in the potential application of β-mannanases has increased in several industrial processes because of their important role in the bioconversion of lignocelluloses, one of the most abundant reusable resources in nature.

Additionally, laminarinase is another cold-loving enzyme that is widely spread throughout bacteria, archaea and eukaryotes. To our knowledge, laminarinase has not been reported from yeast until now. The enzyme plays essential roles in the degradation of microbial saccharides by hydrolysing the β-1,3-linkages of glucans and, therefore, is crucial for nutrient uptake and energy production in these microorganisms. The enzyme has received increased attention due to its potential use in several biotechnological applications, including industrial processes, food industries, and bioremediation.

The genome and proteome study revealed structural characteristics of organisms and facilitates to simulate, predict and infer functional properties of genes. The following main question has to be answered in this study:

(i) How different glycosyl hydrolase family enzymes have been adapted to cold temperature?

1.4 Research Goal and Objectives

The main goal of this research is to study psychrophilic adaptation of Glycosyl hydrolase enzymes from the psychrophilic *G. antarctica* pI12 yeast. To achieve this goal, following objectives have to be met:

(i) To model the structure of two novel Glycosyl hydrolase family enzymes from the psychrophilic yeast *G. antarctica* pI12 by comparative modeling.

(ii) To study and analyze interactions between the chosen Glycosyl hydrolase catalytic enzymes and the substrates.

(iii) To study cold adaptation of the chosen Glycosyl hydrolase family enzymes from the psychrophilic yeast *G. antarctica* pI12 based on primary sequence, structure analysis, and molecular dynamics simulation.

(iv) To investigate and establish novel strategies used by psychrophilic Glycosyl hydrolase family enzymes from the psychrophilic yeast *G. antarctica* pI12 to adapt with cold environment.

1.5 Scope of the Study

The research involves psychrophilic adaptation study of glycosyl hydrolase enzymes using *in silico* approch. In this work, two genes were selected belonging to *G. antarctica* including β-mannanase and β-glucanase, and subjected to comparative modeling.

In order to effectively identify the cold adaptation mechanisms of glycosyl hydrolase enzymes, all analysis were performed comparatively using mesophilic, thermophilic, and hyperthermophilic counterparts of the selected genes. The 3D structure of the genes was further subjected to docking and molecular dynamics simulations and different structural and functional characteristics were studied via MD simulations.

1.6 Thesis organization

The thesis is organized in the following chapters:

Chapter 1 describes the research outline. It presents background of the study and problem statement. In sequel, the research goal and objectives are explained and scope of the research is discussed.

Chapter 2 includes a review on literatures related to the study. As preliminary, basic concepts of the related subjects are described, and then, the chapter moves to description of related studies on research area. Finally, the recent trends of the research are explained.

Chapter 3 presents the research methodology of this research including the operational framework of the research to reach the main objectives. Furthermore, the required methods and materials in this research are described.

Chapter 4 shows the results of the structure prediction and cold adaptation study of a novel laminarinase (3.2.1.6, endo 1,3(4) β-glucanase). It includes the results obtained from the conducted experiments and discussions related to the defined objectives.

Chapter 5 shows the structure prediction results and cold adaptation analysis of a novel mannanase (3.2.1.78, endo 1,4 β-mannanase). The results of conducted experiments and discussions related to the defined objectives are included in this chapter.

Chapter 6 concludes the thesis by a general discussion on the research results. Furthermore, the chapter finally suggests the challenging and emerging trends for the future studies.

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