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.