HOMOLOGY MODELING ANALYSIS OF GSTD3 FROM *DROSOPHILA MELANOGASTER*

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UNIVERSITY OF MALAYA

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ABSTRACT

Glutathione S-transferase is a detoxifying enzyme that responsible in catalyzing the conjugation of electrophilic xenobiotics compound with the thiol group of glutathione by biotransforming it into hydrophilic compound that can be eliminated from the cells via ion channel transport system. In *Drosophila melanogaster*, its GSTs have been divided into many classes in which two of them are organism specific, delta and epsilon. Members of delta GSTs which consist of 10 members are located at 3R chromosome. However, in delta isoform 3, there is truncation of several amino acids including conserved tyrosine residue at positions 5 and 6. Since D3 has been confirmed and observed to be expressed in pre adult development, therefore, I hypothesized that there is other tyrosine residue from different positions that have replaced the missing tyrosine. In order to determine the catalytic residue, homology modeling of D3 was carried out by taking 3EIN as template sequence. The template was obtained from UniProt database provided that stores protein sequences and functional information of the protein. Since I was interested in selecting the template with the experimental determined structure, therefore, 3EIN was selected as the suitable template. This is because, the sequence identity between target and template sequence was 63% whereby the model generated from this value could regarded as a good model. In building the model structure for query sequence, Modeller program command prompt was employed and the model was evaluated by PROCHECK program implemented by PDBSum database. After model evaluation, Swiss-PDBViewer and RasMol were used to visualized, manipulate and calculate the force field energy of the model. 3D structure of D3 showed that it consists of 2 beta sheets which were different from template, which consist of 4 beta sheets. Since GSTs usually work in homodimeric form, therefore, I suggested that D3 paired up with D3 by sharing their beta sheets. In term of catalytic residue, it suspected

that other tyrosine residues from different position had compensated the missing tyrosine of positions 5 and 6. From the model visualization, it shows that side chain from tyrosine of positions 89 and 97 were projecting into the GSH binding site. Referring to the force field energy calculated, it shows that tyrosine of position 97 is more suitable since it has the lowest energy.

ABSTRAK

Glutation S-transferase ialah sejenis enzim detoksifikasi yang bertanggungjawab dalam menjadi pemangkin untuk tindak balas konjugasi bagi menukar kompaun yang bersifat elektrofilik toksik, dengan bantuan kumpulan thiol yang terdapat pada glutation, kepada kompaun yang bersifat hidrofilik yang mana ia boleh disingkirkan daripada sel-sel melalui sistem pengangkutan saluran ion. Di dalam *Drosophila melanogaster*, GSTs telah dibahagikan kepada beberapa kelas di mana dua daripada kelas tersebut adalah khusus untuk organisma, iaitu delta dan epsilon. Ahli-ahli GSTs kelas delta terdiri daripada 10 ahli yang terletak pada kromosom 3R. Walau bagaimanapun, di delta isoform 3, terdapat kehilangan beberapa asid amino termasuk tyrosin yang terpelihara pada kedudukan 5 dan 6. Memandangkan terdapat kajian yang dilakukan oleh para saintis berkenaan D3 di mana mengikut hasil penemuan mereka, D3 adalah sejenis protin yang diekspresikan di dalam genom lalat dewasa. Oleh itu, hipotesis saya mengatakan bahawa terdapat tyrosin dari kedudukan yang berbeza yang telah menggantikan tyrosin yang hilang. Untuk menentukan asid amino yang mana menjadi pemangkin, maka pemodelan homologi D3 telah dijalankan. Rujukan-rujukan asid amino ini diperolehi daripada pangkalan data UniProt yang disediakan oleh NCBI. Memandangkan 3EIN mempunyai persamaan identiti dengan sasaran D3 iaitu sebanyak 63%, oleh itu 3EIN telah dijadikan sebagai rujukan. Bagi membina struktur model untuk D3, pemodel program, iaitu Modeller telah digunakan dan diaplikasi bagi membina model-model untuk D3. Model-model ini telah dinilai oleh program PROCHECK yang dilaksanakan oleh pangkalan data PDBSum. Selepas penilaian model dilakukan, Swiss-PDBViewer dan RasMol telah digunakan untuk menggambarkan, memanipulasi dan mengira daya tenaga model tersebut. Struktur tiga dimensi D3 menunjukkan bahawa ia terdiri daripada 2 lembaran beta yang berbeza daripada rujukan,

yang terdiri daripada 4 helai beta. GSTs biasanya berfungsi dalam bentuk homodimerik, oleh itu, saya menyimpul bahawa D3 akan berpasangan dengan D3 yang lain bagi berkongsi lembaran beta mereka. Berkenaan dengan asid amino yang bertanggungjawab dalam menentukan aktiviti mangkin, ia disyaki bahawa tyrosin lain yang berasal dari kedudukan yang berbeza telah menggantikan tyrosin yang hilang pada kedudukan 5 dan 6. Dari visualisasi model, ia menunjukkan bahawa rantaian sampingan tyrosin pada kedudukan 89 dan 97 telah mengunjur kedalam tapak yang mengikat GSH. Merujuk kepada medan daya tenaga yang dikira, ia menunjukkan bahawa tyrosin di kedudukan 97 adalah lebih sesuai kerana ia mempunyai tenaga terendah.

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List of Symbols and Abbreviations

DmGSTD3 – *Drosophila melanogaster* glutathione S-transferase delta

GST – glutathione S-transferase

GSH – glutathione

MAPEG – Membrane Associated Proteins in Eicosanoid and Glutathione metabolism

LTC_4S – leukotriene C_4 synthase

FLAP – 5 lipoxigenase activating protein

PGESI – prostaglandin E synthase I

cDNA – complementary deoxyribonucleic acid

HCCA – 2-hydroxychromene-2-carboxylate

DDT – dichlorodiphenyltrichloroethane

NCBI – National Center for Biotechnology Information

BLAST – Basic Local Alignment Search Tool

UniProt – Universal Protein Resource

PBO – piperonyl butoxide

3D – three dimensional

PIR – protein information resource

DOPE – Discrete Optimized Protein Energy

List of Appendices