CHAPTER 1

INTRODUCTION

1.1 Overview

Glutathione transferases are detoxifying enzymes that responsible in protecting the cellular macromolecules against the deleterious effects brought by xenobiotics and radical agents (Sheehan et al. 2001). GSTs act by catalyzing the tripeptide glutathione's conjugation into electrophilic substrates (Figure 1.1) (Chronopoulou & Labrou 2009). Each organism, eukaryotes and prokaryotes, have these machinery in defending themselves from these man-made and naturally exist substrates. In *Drosophila melanogaster*, 42 cytosolic GSTs have been identified and classified into six different classes which are *theta, sigma, zeta, omega, delta,* and *epsilon,* (Ranson et al. 2001), in which the latter two classes are the new classes that being introduced. Moreover, *delta* and *epsilon* are crucial as the members of both classes take part in insecticide resistance (Ding et al. 2003). In *D. melanogaster*, 10 members of delta classes GST have been identified and annotated as DmGSTD1 until DmGSTD10. However, in GST delta-3 (annotated as DmGSTD3), several numbers of N-terminal amino acid are truncated, together with the tyrosine residue in position 5 and 6 which are important in determining the catalytic activity in fruit fly.

In order to determine the amino acid that responsible in catalytic activity, homology modeling will be performed to the query sequence. The query sequence is protein sequence of *Drosophila melanogaster* glutathione S-transferases Delta – 3 (NP_788656.1). The template sequences will be determined and choose according to the percentage of homology which is above 60%. This is because, within the class of GST, the percentage of sequence similarity is 60%. After the pdb file of target sequence has been obtained, the result will be analyzed to find out the amino acid residue in active site.

In this research project, the research project will be divided into four categories which are Chapter 1: Introduction, Chapter 2: Literature Review, Chapter 3: Methodology, and Chapter 4: Results and Discussion.

In Chapter 1, a depth introduction of glutathione S-transferases, its functions, responsibilities in fruit flies, including the different types of glutathione S-transferases will be presented together with the objectives of this research. Chapter 2 will discuss the recent researches that have been carried out in this topic. Meanwhile, the methodology and procedures used for conducting data collection and analysis will be presented in Chapter 3. The Chapter 4 contains analysis and presentation of the results as well as discussion of the results obtained.

1.2 Cell detoxification and function of glutathione S-transferases

Xenobiotics substrates are chemical species, either man-made nor naturally exist, may cause negative effects towards organisms. Xenobiotics is defined as toxic compounds and microbial chemicals, such as plant and fungal toxins, which are introduced and unexpectedly encountered in the food, drugs, soils, pesticides, and organic pollutants (Goff et al. 2006). The effects that brought by these disturbers may deleteriously interact with the organisms (Sheehan et al. 2001) and indirectly produce an unbalance ecosystem. Several of defense strategies and mechanisms have been embraced by cells to overcome the effect by xenobiotics substrates such as sequestration, scavenging, and binding. Despite of all those strategies, catalytic biotransformation was the appropriate option for organisms for protecting themselves against toxic chemical species (Sheehan et al. 2001) which is glutathione S-transferase, that mostly abundance in vertebrates and invertebrates organisms (Alias & Clark 2007).

Glutathione transferases or known as glutathione S-transferases (GST) are detoxifying enzymes that responsible in catalyzing the conjugation of tripeptide glutathione into electrophilic substrates (Chronopoulou & Labrou 2009). Residing mostly in cytosolic part of cells, this enzyme will detoxify the lipophilic, activated, and non-polar xenobiotics metabolites (Sheehan et al. 2001) into less toxic and more water soluble, which in turn can be eliminated easily from the cell (Tang & Tu 1994). With the presence and roles of glutathione transferases in the cell, the cellular macromolecules will be protected from the oxidative assaults (Pettersson et al. 2005) including plant and fungal toxins. Known as the major player in phase II of enzymic detoxification, glutathione S-transferases also responsible in various functions including drug metabolism, removing the reactive oxygen

species, proteins regeneration which is S-thiolated proteins, and others, which led to the research in the field of medicine, agriculture, and biotechnology (Toung et al. 1993).

There are three phases of enzymic detoxification of biotransforming the xenobiotics and radical particles inside the cellular macromolecules (Figure 1.2). In phase I (catalyze by cytochrome P450) and phase II (catalyzed by GSTs), the non-polar and lipophilic xenobiotics will be biotransforming and catalyzing into more water soluble and less toxic which can easily be eliminated from the cell. Meanwhile, the phase III of enzymic detoxification is regarding the elimination of glutathione conjugates from the cell via several pumps such as ATP-dependent GS-X pump (Ishikawa 1992), multispecific organic anion transporter (Heijn et al. 1992), dinitrophenol S-GSH conjugates (Saxena et al. 1992), and multidrug-resistance-associated protein (Jedlitschky et al. 1994). Apart from conjugating the xenobiotics into endogenous water-soluble substrate, glutathione S-transferases also catalyzed the nucleophilic aromatic substitutions known as Michael additions to the reaction of α, β – unsaturated ketones and epoxide ring, which results into the formation of GSH conjugates which are reduced glutathione, UDP-glucuronic acid or glycine, and reduction of hydroperoxides leading to formation of oxidized glutathione (GSSG).

Glutathione S-transferases promote the inactivation and degradation of a wide range of compounds by conjugating the thiol group from glutathione into compounds with electrophilic center (Low et al. 2007). The important factor that determine the catalysis in GSTs is the active residue; serine, tyrosine, and cysteine; will catalytically interacts with the thiol group of GSH and reducing the value of pK_a sulfydryl group from 9.0 to approximately about 6.5 (Chronopoulou & Labrou 2009).

The roles of GST are binding GSH with the xenobiotics substrate (RX) at active site of the protein by bringing that electrophilic substrate to the side of glutathione as well as activate the sulfhydryl group on GSH to allow the nucleophilic attack of GSH on the substrate (Armstrong 1997). Other than this general reaction, glutathione also involved in various types of catalytic reaction including *cis-trans* double bond isomerization of delta(5)-androstene-3,17-dione, and hydroperoxide reduction with cumene hydroperoxide.

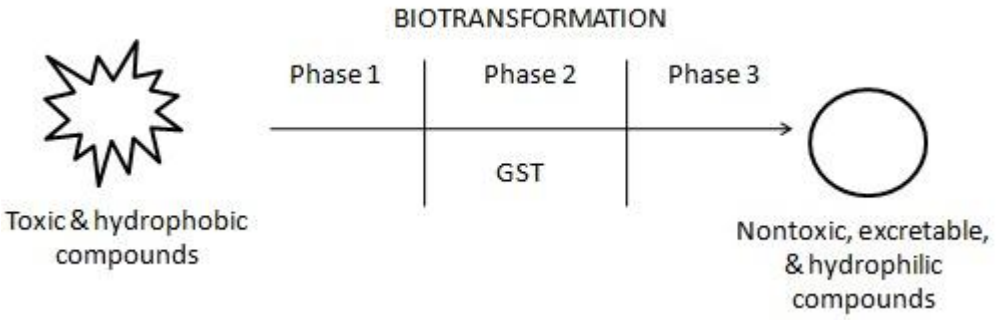

Figure 1.1: Biotransformation of electrophilic substrate into hydrophilic compounds.

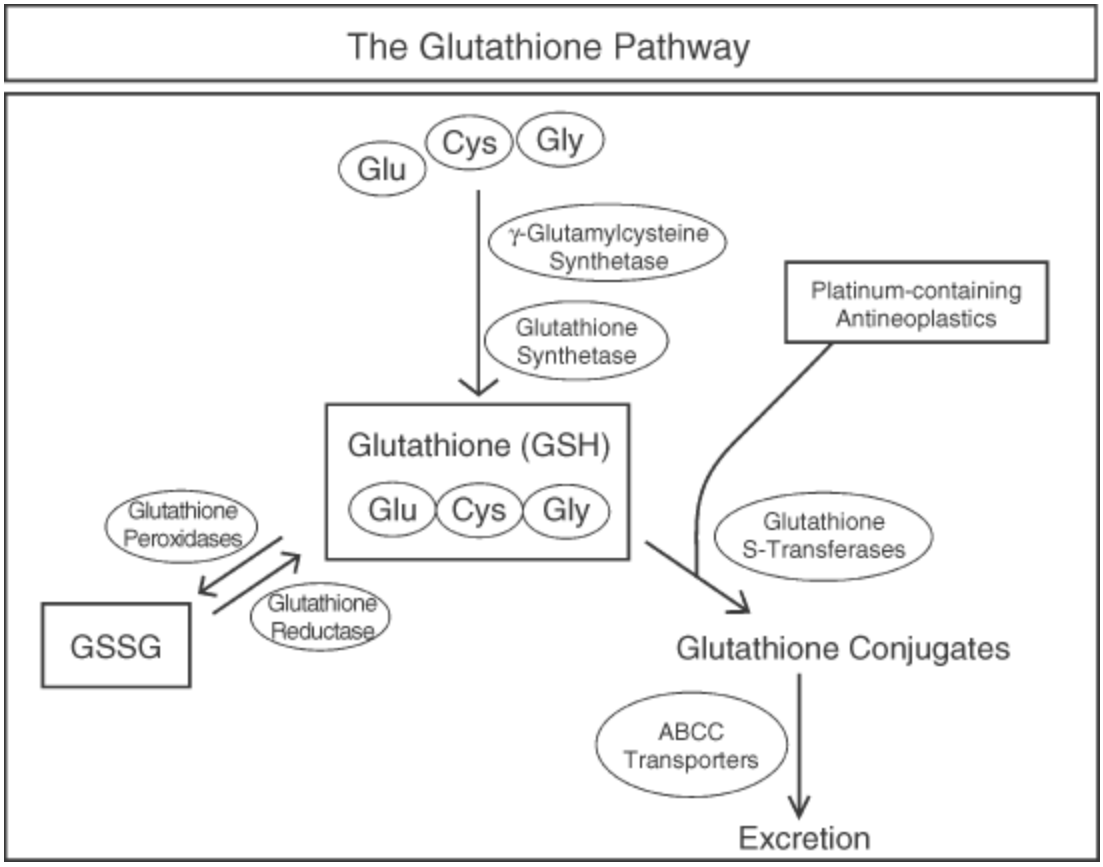

Figure 1.2: Schematic representation of mechanism of glutathione S-transferases.

1.3 Classification of glutathione S-transferases

Glutathione S-transferases are divided into three parts according to their locations which are cytosolic GST, mitochondrial GST, and microsomal GST. The structures are either homodimers or heterodimers. The cytosolic GSTs can be found mainly in mammalian, bacteria, plants, fungi, and insects, with addition of some classes in certain organism whereby the structure consists of two polypeptide chains which forming homodimeric or heterodimeric proteins with molecular weight of approximately 25kDa (Huang et al. 2011). The subunit in cytosolic GSTs contain N-terminal domain and C-terminal domain in which both of them are responsible for GSH binding site (G-site) and hydrophobic substrate binding pocket (H-site), respectively (Huang et al. 2011) whereby both of them were made up from four beta sheets (Chronopoulou & Labrou 2009). Furthermore, the catalytic activity and active site residues of GST are determined by N-terminal. Tyrosine, serine, and cysteine are active site residues that associated with GSTs classes of organisms.

In each organism, the genes in GST gene family were further subdivided into different groups by referring to their amino acid similarities, three dimensional and four dimensional of structural properties, immunological and kinetic properties (Chronopoulou & Labrou 2009). The percentage of sequence similarities among members of the same class are more than 60% whereas members of different class share less than 30% of sequence identity (Sheehan et al. 2001).

1.3.1 Cytosolic GSTs

Cytosolic GSTs is subfamily enzymes that been ubiquitously found in all aerobic organisms in which in human and mammalian there are 15 – 20 different cGSTs (Hayes et al. 2005), in plants there are 40 – 60 cGSTs (Soranzo et al. 2004), 10 – 15 cGSTs in bacteria (Vuilleumier & Pagni 2002), and more than 10 cGSTs indentified in insects (Ranson et al. 2001). Cytosolic GSTs have been categorized into nine superfamilies which are *alpha, beta, delta, theta, mu, pi, sigma, phi,* and *omega* (Figure 1.3). With the average length of 200 – 250 amino acids, cytosolic GSTs active in the form of homodimers or heterodimers with molecular weight of 23 – 30 kDa. It is divided into two domains; Domain I and Domain II; the three dimensional structure of all soluble (cytosolic) GSTs proteins show structural conservation, although they have low level of sequence similarities across the classes (Sheehan et al. 2001).

The domain I, which is N-terminal domain that adopts thioredoxin-like fold, contains four β-sheets that grouped and flanked on each side by three α-helices (Board et al. 2000). The fold constitutes of two structural motifs; N-terminal and C-terminal motifs with arrangements of βαβ and ββα respectively, which linked together by an α-helix identified as α_2 (Figure 1.4). The cis-proline loop at residue 53 is conserved in all cytosolic GSTs as it is important and crucial in maintaining the hydrogen bonding between the protein and GSH substrate (Matthew et al. 1995). Meanwhile, the C-terminal domain (domain II) consists of seven α-helices in which five of them are common to GSTs superfamily and the rest of them would fold back over the top of N-terminal domain which makes the structure of the GSTs superfamily being difference (Matthew et al. 1995). The position of C-terminal domain is located at downstream of thioredoxin structure and is linked with the domain I via short linker sequence (Frova 2006).

Figure 1.4 shows the schematic diagram of thioredoxin folds that employed by N-terminal domain I. It starts with N-terminal motif of β_1 strand followed by α_1 helix and continued with β_2 strand which both of them are parallel to each other and the N-terminal motif is connected to C-terminal motif via α_2 helix. The C-terminal motif, on the other hand, consists of two sequential β-strands (β_3 and β_4) that are antiparallel to each other and followed by a α-helix identified as α_3 at the C-terminus. Those four β-sheets are located on the same plane, while the α_1 and α_3 are located below that plane and α_2 located above the plane. The cis-pro loop which connects α_2 and β_3 has cis conformation that is conserved in all cytosolic GSTs and plays a major role in maintaining the catalytic activity of the protein (Allocati et al. 1999).

Meanwhile, the domain II which begin at C-terminus constitute α-helices in which the amount of α-helices depending on the GSTs classes. For example, Mu and Pi classes have five α-helices whereas Alpha class has six α-helices. Domain II is responsible in hydrophobic binding site whereas the domain I is responsible in GSH binding site (Matthew et al. 1995).

Glutathione S-transferases were first identified in mammals in the year of 1960s and currently, their GSTs have been widely and extensively being investigated and classified (Table 1.1). In mammals, seven subfamilies of cytosolic GSTs has been identified as *alpha, mu, pi, theta, zeta, omega*, and *sigma* in which each subfamilies has different criteria that differ them to each other. For example, in *alpha, mu*, and *pi* classes, their primary structure and size are differ to *theta* class. Apart from that, some criterion that take into account are immunobloting; to identify GSTs' expression, kinetic properties of substrate and inhibitor specificity, the active site that determine from tertiary structure, and difference in ability to hybridize into dimers in quaternary conformation (Sheehan et al. 2001). Meanwhile, in insects, six GSTs classes has been identified which are *delta, epsilon, theta, sigma, zeta,* and *omega*. Each of these classes has their own function which is mainly based on xenobiotics detoxification and insecticide resistance (Chronopoulou & Labrou 2009).

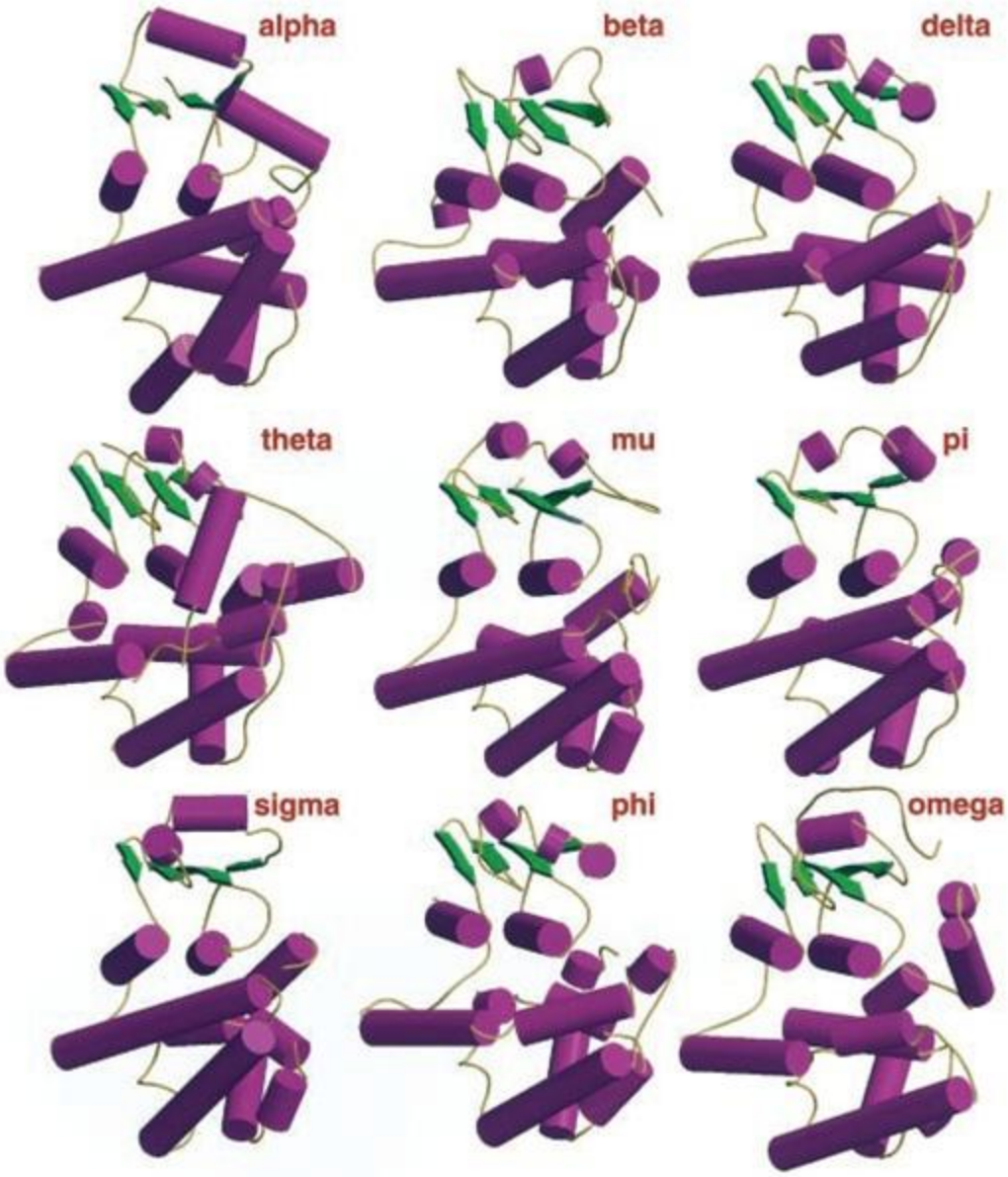

Figure 1.3: Common chain fold of GST superfamily. Helices represented as cylinders and β – strands represented as arrows

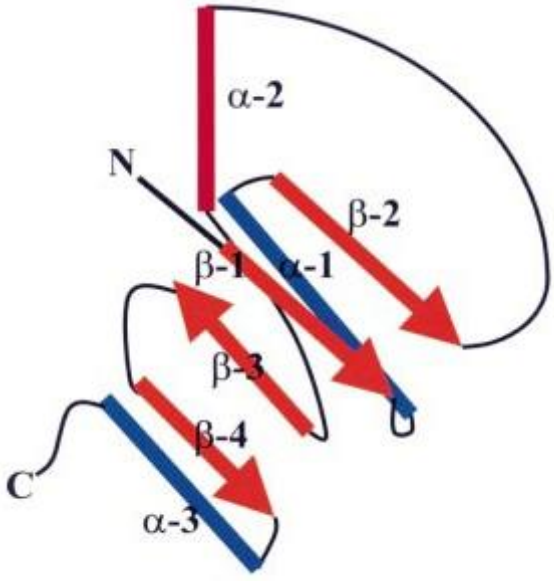

Figure 1.4: Schematic diagram of thioredoxin fold. α-helices depict as cylinders and β-sheets represented as arrows

Table 1.1. List of common and specific cytosolic GSTs

Organism	Common GSTs	Specific GSTs
Mammals	Zeta Theta Omega Sigma	Alpha Mu Pi
Insects		Delta Epsilon
Plant	Zeta Theta	Phi Tau Lambda DHAR
Bacteria	Theta	Beta

1.3.2 Microsomal GSTs

Microsomal GSTs, now known as MAPEG (Membrane Associated Proteins in Eicosanoid and Glutathione metabolism) are also ubiquitous but less numerous than the cytosolic GSTs. Members of MAPEG enzymes involve in the synthesis of eicosanoids, leukotrienes, and prostaglandins which engaged in reactions of catalyzing the GSH substrate. The percentage of similarity between MAPEG and cytosolic GSTs is less than 10% and their proteins subunit are much smaller with amino acid length of 150 amino acids (Frova 2006).

The first microsomal GST being discovered was originated from human and denoted as MGST1. However, the MGST1 does not involve in leukotriene or prostaglandin synthesis but it exhibits the catalytic activities that similar to cytosolic GSTs. The catalyze reactions includes conjugation of GSH into halogenated arenes, polyhalogenated unsaturated hydrocarbons, and reduction of lipid hydroperoxides (Morgenstern et al. 1982). Due to these roles, MGST1 has been categorized as detoxification enzyme responsible in protecting cellular macromolecules from xenobiotics substrates.

Apart from MGST1, new membrane-associated microsomal proteins have been identified. These proteins which involve in biosynthesis of leukotriene include leukotriene C_4 synthase (LTC_4S), 5-lipoxigenase activating protein (FLAP), and prostaglandin E synthase I (PGESI) (Hyun & DeJong 1999). LTC_4S responsible in catalyzing the leukotriene C_4 since it has glutathione transferase activity; FLAP binds non-enzymatically to arachidonic acid for activating an enzyme which later involve in catalyzing the arachidonic acid; whereas PGESI is in charge in reducing cumene hydroperoxide although it has limited glutathione transferase activity (Frova 2006).

The structure of MAPEG shows that it consists of transmembrane domains which are amino and carboxyl termini that protrude into luminal side of membrane. The three dimensional structure suggests that MAPEG is homotrimer whereas quarternary structure of MAPEG reveals that it is non-univocal (Schmidt-Krey et al. 2000).

1.3.3 Mitochondrial GSTs

Mitochondrial GSTs were firstly discovered in rat liver mitochondria in 1991 (Harris et al. 1991), in which this enzyme was initially identified and classified as *theta* class GST due to the result obtained from sequence analysis that showed this mitochondrial GST has limited number of amino acid in N-terminal (Frova 2006). With further experimentation and investigation of its cDNA and protein sequence, there are several differences between mammalian GSTs and mitochondrial GSTs. Therefore, mitochondrial GSTs have been as a new GSTs class termed as Kappa class. Kappa GSTs are dimeric and contains of 226 amino acids in which the amino acid sequences are homologous to *E. coli* DsbA and to bacterial 2-hydroxychromene-2-carboxylate (HCCA) isomerase and, the secondary structure of Kappa GST is more similar to the HCCA and DsbA. Kappa enzymes display GSH-dependent conjugating and peroxidase activity with the substrate which indirectly shares common catalytic features with the other GSTs.

1.4 Glutathione S-transferase in insects

As the insects also experienced pressure from internal and external environment such as toxic substrates, insecticides, and prooxidant plant allelochemicals, it is essential for this xenobiotics substrates being excreted from insects (Mittapalli et al. 2007). Glutathione S-transferases in insects have been purified from more than 24 individual insect species including *Drosophila melanogaster*. These enzymes which are also being expressed at high levels in different isoenzymes forms and at different patterns has been applied as resistance to insecticides (Sheehan et al. 2001). In insects, glutathione S-transferases act by detoxifying the foreign compounds so that these foreign compounds may be excreted easily from the cell by biotransforming them into more water soluble and hydrophilic products.

There are two immunologically classes of insects GSTs which are I and II. The division is based on electrophoretic group in which the first GSTs group is composed of subunits with different isoelectric points while the second GSTs group is composed of polypeptides with acidic pI (Fournier et al. 1992). Class I GSTs can be found in *Musca domestica*, *Drosophila melanogaster*, *Anopheles gambiae*, *Anopheles dirus*, and *L. cuprina* in which according to sequence alignments, this group of GSTs is closely related to GSTs in mammalian Theta class (Sheehan et al. 2001). Class II GSTs, in contrast, are orthologous to mammalian Sigma class, which later then being reclassified as Sigma; and has been discovered in *Manduca sexta* and *D. melanogaster* (Prapanthadaraa et al. 1998). Class I GST is insect specific and therefore being reclassified as Delta. Apart from Delta, a new class defined as Epsilon class is also insect specific in which both of these GSTs classes; Delta and Epsilon; exist in gene clusters in insect genomes as well as the only GSTs that have major role in insecticide resistance (Low et al. 2007). Midgut, fat body, hemolymph,

and other tissues have been identified as the location where the activity of insects GSTs occurred (Che-Mendoza et al. 2009). However, several classes of mammalian GSTs are not included in the classification system for insects GSTs which are Alpha, Mu, and Pi classes.

The importance of enzymes GSTs in insecticide resistance include:

1. Glutathione S-transferases help in detoxification pathway of organophosphorus compounds, cyclodienes, organochlorine, and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane;
2. GSH-dependent route helps in metabolizing the insecticides more efficiently;
3. In some insect resistant strains, there are high levels of activity of GST enzymes;
4. In some strains of insects, the detoxification of xenobiotics compounds are caused by glutathione transferase and ultimately involved in insecticide resistance.

1.5 Glutathione S-transferases in *Drosophila melanogaster*

Fruitfly, or the scientific name is *Drosophila melanogaster*, is an organism that has been extensively studied and investigated by the researchers and scientists. The genome of fruitfly has been sequenced and its genomic data can be retrieved from FlyBase since year 2000. With the size of 139.5 million base pair (Release 5), there are 24 Mb of annotated sequences, 32 pseudogenes, and 13 noncoding RNAs being generated from the heterochromatin of *D. melanogaster* (Smith et al. 2007). The chromosome structure of *D. melanogaster* consists of sex chromosomes X and Y, three autosomes labeled as 2, 3, and 4 in which the autosomes 2 and 3 are subdivided into left and right arm chromosomes (Celniker & Rubin 2003).

42 cytosolic GSTs have been identified from *D. melanogaster*, and these enzymes are classified into six putative families of GSTs which are *delta, epsilon, theta, sigma, zeta,* and *omega*. These enzymes are purified from the developmental stages of *D. melanogaster*. Among those three developmental stages which are larvae, pupae, and adult, the highest specificity of GSTs enzymes occur at the larvae and pupae stages, whereas adult stage exhibits the lowest specific activity (Hunaiti et al. 1995). The differences in terms of enzyme specificity are due to presence of different detoxifying enzymes in each developmental stages and different preferences towards different xenobiotics substrates (Hunaiti et al. 1995). Of those six GSTs families, only two classes are important as they are insect specific and involved in insecticide resistance which are Delta and Epsilon (Table 1.2).

In Delta class GSTs of *D. melanogaster*, there are 10 members denoted as DmGSTD1 until DmGSTD10 in which Dm represents *Drosophila melanogaster*, GST represents glutathione S-transferases, D represents Delta, and number represents the order of discovery. The location of sequences that denotes glutathione S-transferases are located at chromosome 3R (Figure 1.5). DmGSTD1, DmGSTD9, and DmGST10 were located on the same DNA strand, while the remaining seven GST Delta class genes were located on the opposite strand (Sawicki et al. 2003). The function of each member of Delta class are the same which is they involve in glutathione transferase activity. Undoubtedly, DmGSTD1 also play major role in DDT-dehydrochlorinase activity, while DmGSTD2 and DmGSTD8 involve in glutathione peroxidase activity.

In terms of catalytic activity, all members of Delta class in fruitfly exhibit conjugating activity with 1-chloro-2,4-dinitrobenzene except GSTD3 whereas when 4-hydroxynonenal was introduced to the enzymes, only six of Delta members were able to conjugate 4-HNE except GSTD4, GSTD5, GSTD6, and GSTD8 (Sawicki et al. 2003). Prior to this, DmGSTD3 and DmGSTD7 were hypothesized of being pseudogenes; however, since both of them have uninterrupted open reading frames and active towards 4-HNE and CDNB, the former hypothesis could not be accepted. In addition to that, the transcript for DmGSTD3 has been identified by real-time polymerase chain reaction and recombinant proteins in larvae of *Drosophila* that shows GSTD3 is catalytically an active protein.

Nevertheless, the sequences of DmGSTD3 at the N-terminus are truncated of approximately 15 amino acids which also include the crucial and highly conserved tyrosine residue at position 5 and 6. The importance of tyrosine residue is it involves in catalytic activity of GSTs. However, since DmGSTD3 is active towards 4-HNE, this indicates that there is other residues replacing the active residue, tyrosine.

Table 1.2. Summary of *D. melanogaster* GST

GST Class	Putative transcript number
Delta	11
Epsilon	14
Omega	5
Theta	4
Sigma	1
Zeta	2

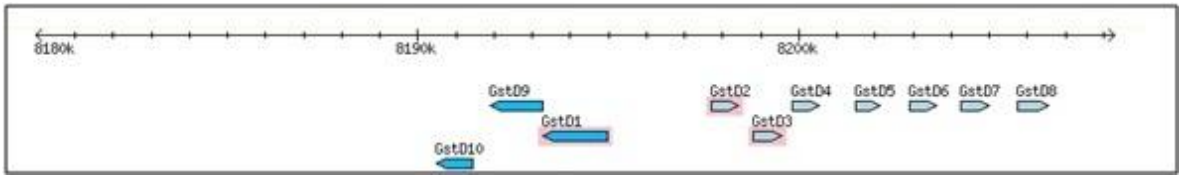

Figure 1.5: Delta class GST cluster

1.6 Structure prediction using homology modeling

Protein structure prediction began in year 1960s in which the most influential prediction method was introduced in the middle of 1970s which called as Chou-Fasman method. This method basically relies on the probabilities of frequencies of each amino acid appeared in different forms of protein structure and using those probabilities the secondary structure of target protein can be predicted (Singh et al. 2012). The significance of Chou-Fasman method in predicting and determining the secondary structure of a protein is alleged to be about 50 – 60% of accuracy. Methods in predicting the protein structure can be divided into three different types which are homology modeling, threading, and *ab initio*.

1. Homology modeling also known as comparative modeling is refers to the process of determining and predicting the three-dimensional structure of an unknown protein by using the amino acid sequence and three-dimensional protein structure which is experimentally known and homologous to the target protein. In homology modeling, the target sequence and template sequence are usually originated from the same ancestor, and although evolution may cause changes in protein sequence, yet their structural and functional properties can be similar due to the presence of conserved amino acids.
2. Threading method is applied whenever there is no homologous protein being discovered. It searches through the whole databases for proteins that have short sequences with similarity towards the target protein. Same as homology modeling, threading only can be done when there are proteins with similar sequences and determined structures.

3. *Ab initio* is derived from *latin* words which applied in bioinformatics in predicting the biological features of a protein using the information of chemical and physical properties of the target amino acid sequence.

Currently, there has been variety of new methods, algorithms, and tools that were developed in assisting the researchers and students for predicting the structure of protein. ExPASy Proteomics Server is an example of bioinformatics resource portal that provide varieties of scientific resources, software tools, and databases in life sciences. The scientists and researchers, therefore, may access to a wide variety of resources in different area in life sciences such as proteomics, genomics, biostatistics, system biology, and others. Examples of tools provided in secondary structure prediction domain are JPred, JUFO, PSA, and PSIpred. PSIpred is a tool for predicting the secondary structure that uses two feed-forward neural networks that analyze the output generated from PSI-BLAST in which the accurateness of result produced by this tool is 80.7% (London 2011).

Other than that, in comparative modeling, a number of tools, programs, and downloadable software are available for the researchers in predicting the three-dimensional structure of a protein sequence. The prediction is based on sequence alignment between target and template sequences. The steps involve in protein prediction are fold assignment, target-template alignment, building of the model, and model evaluation (Marti-Renom et al. 2000). Since the information about proteins have been known, and there are lots of protein sequences being generated from different organisms as well as due to modeling software that have been improvised, as a result, the researchers are able to modeling the protein sequence in which the accurateness of protein prediction is undisputable.

There are varieties of tools and software applicable in comparative modeling of the tertiary structure of a protein such as Modeller, Swiss Model, HHPred, and others. Modeller is a standalone program that applies Python language which is used in homology modeling of the protein structure. The files needed in using Modeller are target-template alignment file, atomic coordinates of the template which is pdb file, and a numbers of script files in Python language. Besides from comparative modeling, Modeller can also perform additional tasks as well such as fold assignment, alignment of protein sequences, and others.

The result produced by Modeller is depend on spatial and stereochemical restrains which is distances and dihedral angles obtained from target-template alignment, bond length and bond preferences, dihedral angles and distances of non-bonded atoms, as well as other restrains which are obtained manually. All of these restrains are attained from different sources including target-template alignment file, CHARM-22, known protein structures files, NMR spectroscopy, topology of secondary structure of a protein and others.

1.7 Research Objectives

Glutathione S-transferases are family of enzymes of that responsible in cellular detoxification of xenobiotics and toxic substrates present in the cellular molecules. As described, GSTs which made of tripeptides act by catalyzing the conjugation of thiol group from glutathione into electrophilic compounds. Subsequently, these electrophilic compounds will prompted into more water soluble and less toxicity in which they can be eliminated from the cell easily.

The genome of *Drosophila melanogaster* has been completely sequenced in 2000. It has been extensively studied and served as model system for eukaryotes. The availability of the complete genome sequence of fruit fly has enabled the developmental and cellular processes of eukaryotes mostly human, to be examined. Therefore, *D. melanogaster* has become an important tool in studying the physiological processes and cellular functions. Currently, the availability of 3D structures are only restricted to GSTD1 and GSTD10.

The major interest of my research project are to develop the three dimensional structure of *D. melanogaster* GST class Delta – 3 and identify the active residue in DmGSTD3. Since the protein sequence of DmGSTD3 has truncation of 15 amino acids at N-terminal, which also includes the conserved tyrosine residue that liable in catalytic activity, I would like to identify the amino acid residue that responsible in determining the catalytic activity of DmGSTD3. In doing that, I need to search for the template sequence that is homologous to the query sequence (DmGSTD3). Although 25% of sequence identity has considered as homologous, however, I decided to choose template sequences of having more than 50% sequence identity with the query sequence. This is because, more than 50% sequence identity may produce a better and reliable model (Sander & Schneider 1991).

In short, the research objectives are:

1. To construct the three dimensional structure of *Drosophila melanogaster* glutathione S-transferase Delta 3 gene using Modeller,
2. To predict the active residue that responsible in determining the catalytic activity of DmGSTD3.