CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

One of the Delta member of *D. melanogaster* known as DmGSTD3 is an intronless active protein. However, the amino acid sequences of GSTD3 with length of 199 amino acids were truncated at N-terminus with approximately 15 amino acids. The amino acids truncation also causing the highly conserved tyrosine residue that involve and important for determining its catalytic activity to be missing. In other organisms such as *Anopheles dirus*, the active residue involve in GSH-conjugation is serine at position 11. However, in other members of Delta class *D. melanogaster*, the active residue that determines the catalytic activity is hypothesized to be tyrosine. Since there are truncations of several amino acids in N-terminal, in which it includes as well the active residue, the assumption is, there is other amino acid at nearby position that responsible in catalytic activity. Apparently, when chemical compounds being introduced deliberately and undeliberately into the cell, there are reactions towards it such as increase and decrease in the expression of GSTs activity. The chemical compounds that have been investigate include Phenobarbital, 1,1-dimethyl-4,4'-bipyridilium, and others.

2.2 Types of chemical treatments being used in insecticides and modes of actions

The activity of GSTs has increased due to tendency of resistance towards insecticides. Major classes of insecticides that have been used in industries nowadays include organochlorine insecticides, organophosphorus insecticides, pyrethroid insecticides and others. Those insecticides cause pain by bombarding the nervous system of target organism. The mechanisms of action for each class of insecticides are in different route

although they basically act on nervous system. Organochlorine acts on the actions of ions over the nerve cell membranes by changing the direction, organophosphate operates on the synapses by disrupting the process of transferring the signals between one cell nerves to other nerves (Davies et al. 2007). Pyrethroid insecticides, on the other hand, act by modifying the normal neuronal function in which it hinders the movements of ions across the nerve cell membrane. On top of that, it also alters the concentrations of calcium ion in the cell. Examples of classes of insecticides discuss above are DDT, methyl parathion, and cypermethrin respectively.

DDT stands for dichlorodiphenyltrichloroethane, has been discovered in 1939 by the Swiss chemist. It is appeared to be an ideal insecticide as it is harmful to the insects, but at the same time is harmless towards human. As mention earlier, DDT cause harm towards peripheral nervous system as initially during the first contact of DDT, the muscles will start to twitch (Low et al. 2010). In the long run, it may lead to paralysis and eventually, death. The DDTase activity has been firstly identified in houseflies, and later on, fruit flies also exhibit the DDTase activity. In DDTase activity, chlorine atoms from DDT are removed in order to produce non-toxic DDE. However, due to prolong used and exposure of DDT towards pests, the pests has developed resistance to the DDT.

Methyl parathion is an organophosphate insecticide that eliminates the pests via digestion system mainly triggers poisoning in the stomach. It is used in controlling the amount of population of insects especially pests in crops including cereals, fruits, vegetables, and others. Methyl parathion, which is an organophosphate, contains chemical substrates were appeared to be susceptible towards conjugation of glutathione metabolism in two mechanisms which are dealkylation and dearylation (Davies et al. 2007).

Cypermethrin is one of the examples of synthetic pyrethroids. Pyrethroids is an artificial compound that pharmacologically active in insecticides. It is formed from a substance known as pyrethrum, and consequently, synthetic pyrethroids were naturally occurred or formed. The percentage of synthetic pyrethroids as insecticides, which applied in agricultural activity, is approximately 25%. This percentage shows that insecticides made of synthetic pyrethroids were acceptable by the society and users. Furthermore, the properties of synthetic pyrethroids had enabled them to be used extensively by the users neither in commercial way nor in agricultural way. Their features include high specificity towards pest and at the same time low level of toxicity towards human.

2.3 Effects of insecticides towards glutathione S-transferase

Mechanism and reaction of insecticides towards glutathione S-transferase were varied depending on type of insecticides, mode of actions, and level of toxicity. However, evolution that occurred inside the pests, mainly insects, had created major problems towards agricultural industries. This evolution which eventually enhanced the detoxification mechanisms of insects had caused the insects to become more resistance towards the insecticides.

DDT dehydrochlorinase activity was first discovered in *Musca domestica* (housefly) and ultimately discovered in other organisms as well including *Drosophila melanogaster, Anopheles gambiae, Anopheles dirus,* and *Aedes aegypti.* The mode of action of DDT dehydrochlorinase activity is a chlorine atom from DDT was removed in order to form nontoxic DDE (1,1-bis-(4-chlorophenyl)-2,2-dichloroethene which is then it can be eliminated later by targeting it to the ion transporter. Despite the discovery of DDT-ase activity inside the organisms, there were also other different glutathione S-transferase

implicated in that resistance towards DDT insecticides. In *Drosophila melanogaster*, DDT dehydrochlorinase activity has been associated in GST class Delta isoform 1 (Tang & Tu 1994). As reported by Tang and Tu, the activity of DDT dehydrochlorinase was occurred in GSTD1 from fruit flies in which more than 30% amount of DDT were biotransformed into DDE. They also reported that the homologs of GSTD1 of *Drosophila* may also exhibit the DDT dehydrochlorinase activity as in GST isoform 1 of *Musca domestica.*

Regarding the glutathione S-transferase Delta class isoform 1 also, apart from being expressed during DDT dehydrochlorinase activity, it also shows positive reactions towards paraquat, 4-phenybutyrate, phenol, and pentobarbital. Apart from that, GSTD1 isoform demonstrates responsiveness towards phenobarbital and paraquat treatments (Alias & Clark 2007). As reported, the insects, fruit flies, were treated and exposed with those two toxic chemicals throughout different stages which were larval development, pupation, emergence, and adult maturation. From this experiment, D1 isoform exhibit positive reaction as its volume was tremendously increased by 190% for paraquat and 40% for phenobarbital (Alias & Clark 2007).

Meanwhile, in phenobarbital treatment, all of the Epsilon class was expressed in which Epsilon isoforms 3 and 6 demonstrate greater increase of more than 50% from initial volume. During the treatment of paraquat also, there were other members of GSTs isoforms have been identified in which the amounts of these GSTs inside the organisms were increased. The GSTs that was affected with paraquat were Delta class of isoform 1 as well as Epsilon isoforms 6, 7, and 9 (Alias & Clark 2007). Since GST Delta isoform 1 is the most GST expressed in fruit fly, therefore, it involves not only in conjugation of xenobiotics molecules by coupling with ligand, glutathione, but also implicates in other different functions as well. The functions include peroxidase activity and catalyzing the

conjugation of halogenated hydrocarbon substrates as well as ene-als. As the GSTD1 from *Drosophila melanogaster* showed an increase in its concentration, and due to its different functions as well as most expressible GST, therefore, the increase obtained was probably due to toxicological significance.

On the other hand, the activity of expressed GSTs associated with the methyl parathion activity resulted in expression of different types of glutathione S-transferase as well as their concentrations (Alias & Clark 2012). This insecticide has been applied to the fruit flies by exposing them to the medium of methyl parathion and acetone. Meanwhile, in control experiment, the fruit flies were exposed only to acetone medium. From the experiment carried out, they found out that GST class Epsilon class isoform 6 and 7, as well as Delta class isoform 1 showed significant increase in their expression. Apart from GSTs protein, other kinds of proteins were also expressed including dehydrogenases, lactoyl glutathione lyase, and thioredoxin peroxidase.

Cypermethrin, a naturally occurring pyrethrin insecticide from plant *Chrysanthemum cinerariafolium* is used in controlling the population of adult vectors such as houseflies, fruit flies, and mosquitoes (Cetin et al. 2010). With the frequent used of cypermethrin, the pests have developed some resistance against the insecticides. Due to this problem, the agricultural communities have to increase the amount of insecticides to overcome the inhabitants of insecticides. Consequently, the resulted crops become reduced in terms of quantity and quality.

Therefore, some insecticides have been reformulated in which different volumes of the synergist effective substances were added into the insecticide. The purposes of adding this substrate are to increase the effectiveness of the insecticides, extend the duration of

insecticides to pests, and reduce the resistance of pests towards insecticides as well as reduce the amount of insecticides needed to apply to the crops. Piperonyl butoxide (PBO) is an example of effective synergist substance that employed in pyrethrin insecticide. The mode of action of PBO is when PBO is used together with pyrethrin insecticide, it will inhibit the enzymes which responsible in degrading the insecticide molecules and thus, making the insecticide to be more susceptible and effective towards insects (Metcalf 1967).

The adult and larvae fruit flies were exposed towards cypermethrin insecticide and cypermethrin insecticide plus PBO, as control medium and experimental medium respectively. In experimental medium, different rates and volumes of PBO being added and the outcomes of fruit flies towards the medium were observed. From the experiment conducted, they observed that the mortality rate of fruit flies towards cypermethrin plus PBO decrease by time. Also, when the concentration of PBO used increased the mortality rate of adult fruit flies decrease much faster than lower concentration of PBO. However, in control medium, it took about 30 minutes to eliminate 60% of the fruit flies. Therefore, they speculated that, PBO is essential to be used in insecticide as well by taking into account of their concentration. Meanwhile, in larvicidal activity, only 25% of PBO is essential by fully eliminating the larvae of fruit flies (Cetin et al. 2010).

The effects of cypermethrin towards different stages of development of fruit flies also have been studied (Karatas & Bahceci 2009). In this experiment, cypermethrin was added into the culture medium as nutrition. From the experiment, they observed that cypermethrin affected the early stage of fruit flies as the rate of egg development was reduced (Nadda et al. 2005). This is due to unsuitable integration of yolk which causing the incomplete development of embryo. However, there are no effects brought by cypermethrin in adult stage of *D. melanogaster* (Karatas & Bahceci 2009).

2.4 Evolution of GST along *Drosophila* species

Glutathione S-transferase that involves in phase II detoxification enzymes has been associated with insecticide resistance in the insects. In fruit flies itself, GST is responsible in protecting the organism from oxidative stress, toxic metabolites, and others. In 12 species of *Drosophila,* the GST genes may have evolved due to toxins, environments, feeding substrates, and others. In *Drosophila* also, GST classes that are important are delta and epsilon, since these two classes are organism specific. Moreover, they have specific functions and expression patterns that differ from other classes of glutathione S-transferase as well as involved in insecticide resistance.

12 *Drosophila* species involved were *D. melanogaster, D. simulans, D. erecta, D. yakuba, D. ananassae, D. pseudoobscura, D. persimilis, D. willistoni, D. mojavensis, D. virilis,* and *D. grimshawi* in which the strains from *D. melanogaster* was made as reference (Low et al. 2007). From the research of genomic sequences of *Drosophila* species, there were 429 genes and 24 pseudogenes appeared in this family. They found out that *D. ananassae* consist of 45 genes in which most of these genes were members of epsilon class, compared to *D. melanogaster* in which it has 36 GSTs. This gene loss is due to several reasons including long duration of evolutionary time which has caused some genes to become malfunctioning. Other than that, those genes could evolve into having the same functions as other GST genes.

Furthermore, the abundance of GSTs in *D. ananassae* and *D. willistoni*, in which they have 45 and 40 GSTs respectively were because of these two species have greater population sizes. Thus, with larger population sizes, it only permits the evolution of certain selected and small GSTs that occur to be maintained and fixed. In the mean time, species

with lower number of glutathione S-transferase were probably due to decreased in rate of evolution of their gene sequences. Besides that, they also found out that conserved genes tend to cluster at the edge of the gene sequences whereas pseudogenes tend to cluster at the centers of the genes. Since the conserved glutathione S-transferase genes, which were Delta isoforms 1 and 9 as well as Epsilon isoforms 9 and 4 were located at the edge of the gene; they tend to evolve minimally due to having unique function and specific expression patterns. This discovery is corresponding to the function and properties of GST Delta isoform 1 in which Delta isoform 1 is the gene that is most expressible and involve in varieties of functions.

2.5 Protein active site prediction

Active site of a protein is hollows found at the surface of a protein that allows two molecules to be fitted together as in lock and key mechanism. In lock-key model, protein is denoting as lock, whereas enzyme or substrate is denote as key. Usually, only a specific key with specific characteristics, properties, and shapes can fit into the specific lock. Naturally, the importance of a protein is depending on its active site, since it may reveal the functional and structural mechanism of a protein (Chou & Cai 2004). For example, in drug design, the active site of a target protein should be savvy for constructing a better drug.

There are several methods had emerged for predicting the active site of a protein. For example, methods introduced by (Miranker 1991) and (Goodford 1985) suggested that the interaction energies between receptor and different probe were calculated and the most favorable energy was selected as the active site of a protein. However, these methods were difficult to be done because it requires the assignment of locations of protons and their charges to the substrate atoms which were not easy to be done. Thus, to overcome these

problems, nowadays, there are lots of available programs and software produced which assist the researchers and scientists in predicting the active site of a protein including Par-3D, Pocket-Finder, Q-SiteFinder, and others.

Par-3D is a web based tool that applies the theory of active site residues is a conserved feature in functionally and structurally related families. It uses the geometrical parameters of known proteins as a training set to predict the active site of a target protein (Goyal et al. 2007). Pocket-Finder and Q-SiteFinder is another web based tool developed by University of Leeds in which Pocket-Finder is based on Ligsite by scanning the protein. Meanwhile, the Q-SiteFinder works by measuring the most energetically favorable binding sites between protein and van der Waals probes (Laurie & Jackson 2005).