3936661 MBMG/M : MAJOR: MOLECULAR GENETICS - GENETIC ENGINEERING; M.Sc.(Molecular Genetics-Genetic Engineering)
KEY WORDS : GLUTATHIONE S-TRANSFERASE/ INHIBITION STUDY ANOPHELES DIRUS/ ISOENZYME/ ALLELIC FORMS MOSQUITO LARVAE

PEERADA PROMMEENATE: CLONING, EXPRESSION AND CHARACTERIZATION OF CLASS I GLUTATHIONE S-TRANSFERASE ISOENZYMES IN *Anopheles dirus*. THESIS ADVISORS: ALBERT J KETTERMAN Ph.D., SAKOL PANYIM Ph.D., CHANAN AUGSUTHANASOMBAT Ph.D. 120 p. ISBN 974-661-997-7.

Glutathione S-transferases (GSTs: E.C. 2.5.1.18) are a multigene family of multifunctional dimeric proteins that play a central role in detoxication of xenobiotic compounds such as herbicides and insecticides. The GSTs in insects are of interest because of their role in insecticide detoxication. The multiplicity of insect GSTs would predict that they should have many physiological functions due to the broad range of substrate specificity of the enzymes. In this thesis study, five allelic forms of the *Anopheles dirus* GST, adGST1-1, were cloned, expressed in *E.coli* and functionally characterized. They were obtained by using the reverse transcriptase polymerase chain reaction of mRNA purified from *An.dirus* larvae. The soluble form of the 23-kDa cloned GST protein was affinity purified by S-hexylglutathione agarose chromatography via a batch binding method. After the purification step, the enzymes were characterized by inhibition with various kinds of effector molecules which included general substrates and inhibitors for GST classes, insecticides and fatty acids. The inhibition study was used to obtain information about the physical and chemical properties of the amino acids that had been changed between each allelic form of the GSTs. Each allelic form had a different pattern of inhibition with the 16 compounds studied, which indicated the effect of amino acid residues in each position in the catalytic function of the enzymes. An extra 15 amino acids on the N-terminus of one GST recombinant protein effects the enzyme specificity. Based on an available crystal structure of insect GST, several of the residue changes were not in the putative substrate-binding pocket. Therefore these residue changes may modulate the enzyme specificity through alterations in the tertiary structure. The amino acid changes and the N-terminus leader sequence were shown to confer different kinetic properties to the enzymes. The characterization and expression of multiple allelic forms of the GST demonstrate a possible network, existing *in vivo*, of a system of enzymes with an extended range of substrate specificity. Insects possessing this broad spectrum capability for detoxication of xenobiotic and endogenous compounds would have a selective advantage for survival.