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KANYA JIRAJAROENRAT: CLONING, EXPRESSION, AND CHARACTERIZATION OF INSECT CLASS I GLUTATHIONE S-TRANSFERASES FROM *ANOPHELES DIRUS* B. THESIS ADVISORS: ALBERT J. KETTERMAN, Ph.D., CHANAN ANGSUTHANASOMBAT, Ph.D., CHARTCHAI KRITTANAI, Ph.D. 131 p. ISBN 974-664-344-4.

Glutathione S-transferases (GSTs: E.C.2.5.1.18) are a multiple gene family of multifunctional dimeric enzymes which catalyze a board range of substrates and play an important role in detoxication of xenobiotic compounds. The GSTs in insects are of interest because they are involved in insecticide resistance. In this thesis study, three cDNA sequences of glutathione S-transferases: *adgst1-2*, *adgst1-3* and *adgst1-4*, the alternatively spliced products of the *adgst1AS1* gene, were obtained from the 4th instar larvae of *Anopheles dirus* B mosquito by RT-PCR reactions. The nucleotide sequences of these three cDNAs share >67% identity and the translated amino acid sequence share >61% identity. A comparison of the *An. dirus* to the *An. gambiae* enzymes shows the adGST1-2 versus agGST1-4, adGST1-3 versus agGST1-5 and adGST1-4 versus agGST1-3 have 85, 92 and 85% amino acid sequence identity respectively, which confirms that the orthologous isoenzymes occurred across the anopheline species. These three genes were expressed at high levels, approximately 15-20 mg from 200 ml of the *E. coli* culture. The recombinant enzymes were purified by affinity chromatography on an S-hexylglutathione agarose column. The subunit sizes of adGST1-2, adGST1-3 and adGST1-4 are 24.3, 23.9 and 25.1 kDa. The recombinant enzymes have high activities with CDNB, detectable activity with DCNB but markedly low activity with ethacrynic acid and *p*-nitrophenethyl bromide. The adGST1-3 was shown to be the most reactive enzyme from the kinetic studies. Permethrin inhibition of the three enzymes was different being uncompetitive for adGST1-2, noncompetitive for adGST1-3 and competitive for adGST1-4. Despite the enzymes being splicing products of the same gene and sharing identical sequence at the N-terminal domain, these GSTs show distinct substrate specificities, kinetic properties and inhibition properties.