Article

# CHARACTERIZATION OF MOSQUITO CYP6P7 AND CYP6AA3: DIFFERENCES IN SUBSTRATE PREFERENCE AND KINETIC PROPERTIES

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*Cytochrome P450 monooxygenases are involved in insecticide resistance in insects. We previously observed an increase in CYP6P7 and CYP6AA3 mRNA expression in Anopheles minimus mosquitoes during the selection for deltamethrin resistance in the laboratory. CYP6AA3 has been shown to metabolize deltamethrin, while no information is known for CYP6P7. In this study, CYP6P7 was heterologously expressed in the Spodoptera frugiperda (Sf9) insect cells via baculovirus-mediated*

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*expression system. The expressed CYP6P7 protein was used for exploitation of its enzymatic activity against insecticides after reconstitution with the An. minimus NADPH-cytochrome P450 reductase enzyme in vitro. The ability of CYP6P7 to metabolize pyrethroids and insecticides in the organophosphate and carbamate groups was compared with CYP6AA3. The results revealed that both CYP6P7 and CYP6AA3 proteins could metabolize permethrin, cypermethrin, and deltamethrin pyrethroid insecticides, but showed the absence of activity against bioallethrin (pyrethroid), chlorpyrifos (organophosphate), and propoxur (carbamate). CYP6P7 had limited capacity in metabolizing λ-cyhalothrin (pyrethroid), while CYP6AA3 displayed activity toward λ-cyhalothrin. Kinetic properties suggested that CYP6AA3 had higher efficiency in metabolizing type I than type II pyrethroids, while catalytic efficiency of CYP6P7 toward both types was not significantly different. Their kinetic parameters in insecticide metabolism and preliminary inhibition studies by test compounds in the flavonoid, furanocoumarin, and methylenedioxyphenyl groups elucidated that CYP6P7 had different enzyme properties compared with CYP6AA3.* 

**Keywords:** cytochrome P450; pyrethroid; CYP6P7; CYP6AA3; kinetic study

## INTRODUCTION

Cytochrome P450 monooxygenases (P450s or CYPs) constitute a superfamily of heme-containing monooxygenases that play roles in the metabolisms of endogenous and exogenous compounds, including insecticides (Feyereisen, 1999). In insects, increased expression level of P450s, leading to enhanced detoxification of insecticides, is suggested to play a role in insecticide resistance (Feyereisen, 1999; Scott, 2008). A link between insecticide resistance, high level of monooxygenase activity, increased P450 expression, and ability of P450s to metabolize insecticides has been noted in various insects. For instance, an increase in CYP6D1 mRNA and protein expression level has been observed in the Learn Pyrethroid Resistant (LPR) strain of *Musca domestica*, and CYP6D1 microsomal enzyme has been shown to metabolize pyrethroids at a higher level in LPR strain than the susceptible strain (Wheelock and Scott, 1992; Tomita et al., 1995; Zhang and Scott, 1996). Overexpression of CYP6BQ9 and CYP6P3 in association with pyrethroid resistance has been reported in deltamethrin-resistant strain of *Tribolium castaneum*, and permethrin-resistant field *Anopheles gambiae* mosquitoes, respectively, and heterogously expressed enzymes of *CYP6BQ9* and *CYP6P3* demonstrate activities in pyrethroid metabolism (Müller et al., 2008; Zhu et al., 2010).

*Anopheles minimus* is one of the primary malaria vectors in Thailand. We previously selected a laboratory strain of *An. minimus* species A for deltamethrin resistance (Chareonviriyaphap et al., 2002). We observed elevated enzyme activities of mixed function oxidases in the resistant mosquitoes, suggesting that P450s could act as a primary route of insecticide detoxification (Chareonviriyaphap et al., 2003). Further studies demonstrated that, among the CYP6 P450 cDNA fragments obtained, *CYP6P7*, *CYP6P8*, and *CYP6AA3* genes were overexpressed in deltamethrin-resistant mosquitoes (Rongnoparut et al., 2003; Rodpradit et al., 2005). The increase in CYP6P7 and CYP6AA3 transcripts was correlated with increased resistance to deltamethrin in



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