ABSTRACT

Anopheles funestus Giles is a major vector of malaria in Africa and pyrethroid resistance observed in this species has disrupted malaria control in southern Africa. Metabolic detoxification, based on the overproduction of cytochrome P450s, specifically *CYP6P9* and *CYP6P13,* was identified as the principal resistance mechanism in both field and laboratory populations. This project aimed to characterize this resistance mechanism further, both on a molecular as well as a biochemical level. Biochemical analysis on total P450 activity levels revealed a 25.5-fold increase in the resistant strain compared to a pyrethroid susceptible strain. Analysis of the effect of pyrethroids on mRNA expression of three P450 genes showed that two of them (*CYP6P9* and *CYP6P13*) as well as Cytochrome oxidase I (*COI*) was induced. HPLC analysis using a heterologously (recombinant) expressed *CYP6P9* enzyme, showed that *CYP6P9* was able to metabolize the pyrethroid permethrin and that it was catalytically efficient. Immunoblotting revealed no significant variation in *CYP6P9* protein abundance between the different *An. funestus* colonies. Although an approximate molecular weight ($\approx M_r$) of 58kDa was predicted for CYP6P9, two fragments were detected at $\approx M_r$ 52,000 and ≈45,000. The smaller fragment was very likely a result of proteolytic degradation. Statistical analysis revealed there was no significant difference in *CYP6P9* protein expression between strains or sexes. Although *CYP6P9* mRNA is over-expressed it is important to assess the abundance of protein as well when elucidating whether a gene and its protein are important candidates in resistance. Differences in pyrethroid resistant or susceptible profiles of *An. funestus* colonies could be related to enzyme affinity for substrate and stability of *CYP6P9* protein however; it is recommended that further studies need to be done before any conclusions can be drawn. *CYP6P9* in *An. funestus* is a major candidate in conferring pyrethroid resistance and the pyrethroid resistant strain is able to metabolize the pyrethroid permethrin.