Expression Profiles of Tsetse (*Glossina* spp) *Glossina* Proteolytic Lectin (*G*pl) Gene

By

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or any other award

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SUMMARY

Tsetse flies are important agricultural and medical vectors of African trypanosomes, the causative agents of trypanosomosis in humans and animals. The life cycle of the protozoan parasite Trypanosoma, in its invertebrate vector begins when the tsetse fly feeds on an infected mammalian host. An important step in the establishment of tsetse midgut infection involves transformation of bloodstream-form trypanosomes into procyclic forms. This process is mediated by a wide variety of factors, all of which are intrinsic to the tsetse, trypanosome and the host blood. Tsetse midgut factors involved include lectins, trypsin-like molecules, lysins and lectin-trypsin complex. Recently *Glossina* proteolytic lectin gene (*Gpl*) that encodes for a protein with both lectin and trypsin activities in *Glossina fuscipes fuscipes* was discovered. *Glossina* proteolytic lectin induces transformation of bloodstream-forms trypanosomes to procyclic forms *in vitro*. The gene is bloodmeal induced and is expressed in members of *Glossina* species but not in other haematophagous insects. This suggests that it may be playing an important role in the interactions between tsetse and the trypanosomes and hence the vectorial capacity. The present study was undertaken to gain insight into the effect of trypanosome parasites on the expression of *Gpl* gene and how its expression compares in different *Glossina* species.

Quantitative competitive reverse transcriptase polymerase chain reaction (QC-RT-PCR) was used to assess the expression levels of *Gpl* gene in infected and uninfected *G. f. fuscipes* ~~at~~ 24, 48 and 72 h post feeding. Expression levels were also compared in *G. pallidipes* and *G. f. fuscipes* at 0, 24, 48 and 72 h post feeding. Expression levels of *Gpl* gene remained high throughout the digestion cycle following both infective and

uninfective bloodmeal. However, the expression was parasite responsive, with the expression being lower in trypanosome infected *G .f. fuscipes* ($p<0.05$). At 24, 48, and 72 h, infected *G. f. fuscipes* expressed 6.02×10^5; 6.73×10^5 and 7.92×10^5 *Gpl* gene transcripts respectively, while uninfected *G. f. fuscipes* expressed 7.29×10^5; 7.45×10^5; 8.89×10^5 transcripts respectively. Down regulation of *Gpl* gene expression by trypanosomes might be a possible mechanism used by the parasites to survive and establish themselves in the hostile midgut environment.

Expression of *Gpl* gene varied significantly in *G. pallidipes* and *G .f. fuscipes* with *G. pallidipes* expressing lower levels of the transcripts ($p<0.05$). In both species a linear increase in the gene expression was observed with teneral flies expressing low transcript levels. Teneral *G. pallidipes* expressed 4.82×10^5 and *G. f. fuscipes* expressed 6.22×10^5 . At 24, 48 and 72 h, *G. pallidipes* expressed 5.25×10^5; 6.39×10^5 and 7.19×10^5 *Gpl* gene transcripts respectively as compared to 7.29×10^5; 7.45×10^5; and 8.89×10^5 in *G. f. fuscipes.* This trend suggests regulation at transcription level. Higher *Gpl* gene transcript levels in *G. f. fuscipes* may be among the factors contributing to their refractoriness to trypanosome infection as opposed to the susceptible *G. pallidipes.*

Although several factors have been implicated in the successful transformation and establishment of trypanosomes in tsetse, down regulation of expression of *Gpl* gene in refractory *G. f. fuscipes* and the variation of its expression in susceptible (*G. pallidipes*) and refractory (*G. f. fuscipes*) tsetse flies suggest that there is an optimal concentration of *Gpl* required for the establishment of midgut infection. Trypanosomes could be playing a key role in the modulation.