

The salivary complement inhibitor from *Anopheles aquasalis* (Diptera, Culicidae) belongs to the gSG7 family of salivary proteins

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Anopheles aquasalis is an important malaria vector well distributed in coastal areas of South America, including Brazil. As many hematophagous arthropods, it inoculates saliva into host skin during bloodfeeding in order to facilitate the meal as its saliva presents pharmacological activity, such as antihemostatic and immune modulators. We previously demonstrated that *A. aquasalis*' salivary gland extract (SGE) is capable of inhibiting the alternative pathway of human complement system (CS), a major effector of immune response, but the identity of the inhibitor remained unknown. The objective of the current study was to identify this inhibitor in the saliva of *A. aquasalis*. Firstly, *A. aquasalis* SGE and the recombinant salivary complement inhibitors from *A. albimanus* and *A. darlingi* were resolved in SDS-PAGE and silver stained. These recombinant proteins belonging to the gSG7 family act as alternative pathway inhibitors and were expressed in *E. coli* BL21 pLys. The recombinant bands were compared with the *A. aquasalis* SGE band profile. In the lanes with the recombinant proteins, only one band was detected. In the *A. aquasalis* SGE sample, eight major bands were observed, including an intense band with similar molecular weight of the recombinant proteins. In order to investigate if *A. aquasalis* possess a gSG7 salivary protein, we used the *A. darlingi* gSG7 protein sequence and conducted tBLASTn searches on the *A. aquasalis* genome. We encountered a sequence similar to those from *A. albimanus* and *A. darlingi*. CDS and amino acid sequence of putative *A. aquasalis* gSG7 genes were obtained using GeneWise. Orthology relationship among anopheline gSG7 genes and *A. aquasalis* putative gSG7 genes was determined by phylogenetic analysis with MEGA 7. The gSG7 salivary protein from *A. aquasalis* share 85% of identity with the gSG7 salivary protein from *A. albimanus* and 81% identity with *A. darlingi* gSG7. Evolutionary analyses strongly suggests that the *A. aquasalis* gSG7 described here is orthologous to the gSG7 genes of *A. albimanus*, *A. darlingi*, *A. gambiae*, *A. funestus* and *A. sinensis*. The evolutionary tree shows a clear dichotomy between the gSG7 genes from new world and old world anophelines. For confirming the presence of the gSG7 gene in the salivary glands, primers were designed using Primer3Plus software. One day old *A. aquasalis* females were dissected for extraction of salivary glands, midgut and antennae. RNA was extracted and used for cDNA synthesis. PCR reactions were then performed. The reaction confirmed the presence of the gSG7 gene in the salivary glands but not in the other organs of the insect. All together, our results demonstrate that the complement inhibitor in *A. aquasalis* SGE belongs to the gSG7 family of anopheline salivary proteins. This molecule may be involved in the protection of the insects' midgut against CS-mediated damage and could also be involved in the parasite transmission. Granted by: FAPEMIG, INCT-CNPq Entomologia Molecular