

A MALE ACCESSORY GLAND PROTEIN THAT MODULATES FEMALE MOSQUITO (DIPTERA: CULICIDAE) HOST-SEEKING BEHAVIOR

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ABSTRACT. The male accessory gland product that modulates the host-seeking behavior of female *Aedes aegypti* (L.) mosquitoes was found to be a peptide of 7,600 mw. This peptide also prevented subsequent mating behavior and weakly stimulated oviposition. Neither whole glands nor gland fractions from *Anopheles gambiae* had any effect on *Ae. aegypti* females, but those from *Aedes albopictus* were active.

KEY WORDS Mosquito behavior, oviposition, mating, olfactometer, *Ae. aegypti*, *Ae. albopictus*

INTRODUCTION

During egg development, *Aedes aegypti* mosquitoes are often inhibited from seeking a host until after their eggs are laid. This behavioral inhibition is mediated by a neuropeptide, *Aedes aegypti* Head Peptide I, that is released during oogenesis (Brown et al., 1994). An additional modulation of the inhibition results from male accessory gland (MAG) substances that are transferred to the female during mating. Gravid females that have mated are generally less likely to respond to host stimuli than are those that have not yet mated (Fernandez and Klowden, 1995). The inhibition of host-seeking behavior in unmated females can be further increased by injecting them with homogenates of MAGs. We have attempted to isolate the specific substances from the MAG that affect host-seeking behavior and here report their partial purification.

MATERIALS AND METHODS

Mosquito species and rearing

Laboratory stock colonies of *Ae. aegypti* (Linnaeus) UGAL strain, *Aedes albopictus* (Skuse) colonized from the field in Vero Beach, FL, and *Anopheles gambiae* sensu stricto colonized from insects collected in the Kilimanjaro region of Tanzania (courtesy of C. F. Curtis, United Kingdom) were used in our experiments. All species were reared at 27°C and 80% RH under a 14:10 L:D photoperiod. *Anopheles* larvae were fed ground Tetramin[®] fish food, and *Aedes* were fed a standard diet of brewer's yeast, lactalbumin hydrolysate, and finely ground rat chow (1:1:1 by weight). Males and females were separated in the pupal stage and were 3-4 days postemergence when used in experiments. Adults were maintained on 10% sucrose from cotton wicks.

Purification of MAG protein

For each purification, 1,000 MAGs from unmated males were dissected in the cold, homogenized in insect saline (Ephrussi and Beadle, 1936), and centrifuged at 10,000 × g for 10 min at 4°C. The clear supernatant was removed and the proteins were purified in a 2-step gel filtration chromatography. The 1st fractionation was performed by a fast pressure liquid chromatography (FPLC) system (Pharmacia LKB, Pharmacia Co.) for 80 min with a Suprose 12 column (#17-0538-01, Pharmacia). The buffer consisted of 150 mM of ammonium bicarbonate containing 5 mM of potassium phosphate dibasic at pH 7 and a flow rate of 0.5 ml/min. The large number of highly reproducible peaks were combined into 5 fractions that were then dialyzed at 4°C for 12 h and tested with Nessler's solution every 3 h to determine whether any ammonia was still present. Each fraction was then lyophilized in a Speed Vac concentrator for 24 h. For estimation of the proteins, standard markers of blue dextran (mw 2,000,000), thioglobulin (mw 669,000), bovine serum albumin (mw 66,000), and cytochrome C (mw 12,400) were run under identical conditions. For a 2nd purification, the proteins in the active fraction were subsequently purified with a perfusion chromatography system (BioCAD) for 5 min with an anion exchange column (POROS HQ-high capacity strong anion exchange, BioCAD). The buffer used was 25 mM of Tris at pH 7. The eluted fractions were collected manually.

Bioassays of activity

The effects on host-seeking behavior were measured by the procedures described previously by Fernandez and Klowden (1995). In general, each lyophilized fraction was diluted with mosquito saline and injected into 3-day-old unmated females in 0.2 µl of saline that was concentrated to contain the equivalent of protein present in 1 intact MAG. Twenty-four hours after injection, the females were fed on blood, and their host-seeking behavior while

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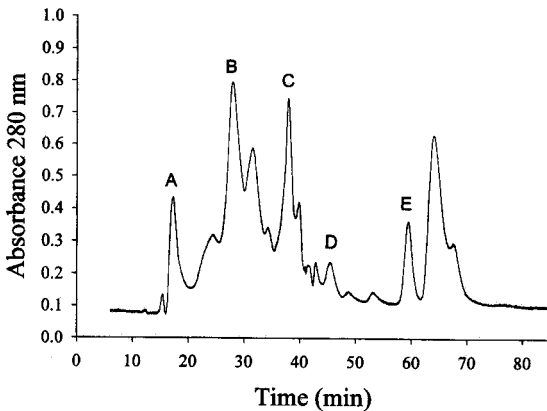


Fig. 1. FPLC fractions (A–E) from the whole male accessory glands of *Aedes aegypti*.

gravid was measured in an olfactometer (Klowden and Lea, 1978) 3 days later. Controls received injections of saline alone.

To determine the effect of purified protein on the inhibition of mating, virgin female mosquitoes were injected with extracts of MAG proteins as above. After injection, 2 previously unmated males were added to each cage with the female and were given the opportunity to mate for a 24-h period. After this period, the females were dissected and were considered to have mated if sperm were present in the spermathecae. Controls consisted of unmated females that were injected with saline alone.

Oviposition behavior was assessed by providing an unmated, bloodfed, and gravid female with an oviposition site after 1st being injected with peptide fractions. The females were examined after 1 day to determine if eggs remained in the ovaries, and the oviposition rate was expressed as the percent-

age of females that oviposited. Controls were bloodfed and gravid mosquitoes that were injected with only saline.

Each of the behavioral experiments initially used 20–30 females, and experiments were replicated 2–3 times. The responses of the experimental and control groups were statistically compared by a Z-test (SigmaStat statistical software, version 1.0).

RESULTS AND DISCUSSION

We identified a 7,600-mw peptide synthesized in the accessory glands of male *Ae. aegypti* mosquitoes that modulates the host-seeking behavior of gravid females. Although the inhibition of host-seeking behavior during oogenesis is induced by a peptide synthesized in neurosecretory cells (Brown et al., 1994), this behavioral inhibition is more likely to occur if the MAG protein is also present (Fernandez and Klowden, 1995).

With the use of FPLC, whole MAGs were 1st separated into 5 fractions (as shown in Fig. 1), each of which was then injected into unmated females that were subsequently bloodfed and developed eggs. About one-third of unmated gravid females normally responded to host stimuli, but the injection of a whole MAG homogenate significantly reduced this response. Of the 5 fractions obtained from FPLC, only 1, peak C, also had a significant effect on host-seeking behavior (Table 1). This same fraction was also responsible for stimulating oviposition behavior in unmated gravid females and had a weak but significant effect on the termination of female receptivity. A 2nd filtration further resolved peak C and yielded 2 additional fractions, A and B. Of these, only peak A significantly inhibited host-seeking behavior when injected into gravid females (Table 2). This fraction also stimulated oviposition but not as effectively as the less-purified

Table 1. Effect of the injection of various male accessory gland fractions from Fig. 1 on the host-seeking, oviposition, and mating behaviors of gravid female *Aedes aegypti* mosquitoes.¹

Fraction injected	Percentage of females \pm SE		
	% host-seeking (n)	% ovipositing (n)	% mating (n)
Saline	24.5 \pm 4.1 (137)	0 (80)	100 (60)
<i>Ae. aegypti</i> MAG homogenate	0* (83)	67.4 \pm 6.7* (49)	6.0 \pm 3.4* (50)
<i>Ae. aegypti</i> peak A	31.3 \pm 4.8 (91)	ND	ND
<i>Ae. aegypti</i> peak B	28.1 \pm 4.7 (93)	ND	ND
<i>Ae. aegypti</i> peak C	4.0 \pm 1.8* (126)	64.6 \pm 5.9* (65)	60.7 \pm 6.3 (61)
<i>Ae. aegypti</i> peak D	34.30 \pm 4.9 (92)	ND	ND
<i>Ae. aegypti</i> peak E	30.8 \pm 4.8 (92)	ND	ND
<i>Aedes albopictus</i> MAG homogenate	0* (23)	100* (39)	ND

¹ An asterisk indicates a significant difference from saline-injected controls. ND = not determined; MAG = male accessory gland.

Table 2. Effect of the components comprising peak C on the behavior of unmated gravid mosquitoes.¹

Fraction injected	Percentage responding to host \pm SE (n)	Percentage ovipositing \pm SE (n)
Saline	34.4 \pm 6.4 (55)	0 (46)
MAG homogenate	0.0* (73)	72.7 \pm 7.8* (33)
A	5.3 \pm 3.0* (56)	30.9 \pm 7.1* (42)
B	23.6 \pm 6.3 (46)	ND

¹ Asterisks indicate significant differences from saline-injected controls. ND = not determined; MAG = male accessory gland.

preparations (Table 2). An electrophoretic analysis yielded a single band corresponding to a peptide of approximately 7,600 mw.

A similar treatment of the MAGs from *An. gambiae* yielded proteins of similar molecular weight, but these had no effect on either the host-seeking or oviposition behavior of female *Ae. aegypti*, nor did whole MAG homogenates from this species. In contrast, those from *Ae. albopictus* both inhibited the host-seeking of *Ae. aegypti* and stimulated oviposition (Table 1). Schmidt et al. (1993) reported that the sex peptides from *Drosophila melanogaster* and *Drosophila suzukii* elicited rejection behavior in the presence of males and an increased egg laying in virgin females of both species. Interestingly, accessory gland extracts of *D. suzukii* could not stimulate ovulation upon injection into unmated *Drosophila pulchrella* females (Fuyama, 1983) although both species belong to the same species subgroup. It has been claimed that this may be the basis of a reproductive isolation between species. However, Schmidt et al. (1993) suggested that the amount of *D. suzukii* male gland fluid transferred may be sufficient to induce the postmating responses in a mating with a female of its own species but insufficient in a heterospecific mating.

In some insects, MAG substances provide the female with nonspecific precursors that may be used for egg development or basic metabolism (Klowden and Chambers, 1991). However, as was previously shown for *Drosophila* (Chen and Balmer, 1989), the effects of these substances in mosquitoes are also mediated by specific peptides not necessarily shared by all species.

Previous attempts to identify the MAG components that affect mosquito mating and oviposition behaviors yielded peptides estimated at 30,000 and 60,000 (Fuchs et al., 1969), between 50,000 and 100,000 (Williams et al., 1978), and approximately 2,000 (Young and Downe, 1982). In the case of *Drosophila*, the molecular weight of the MAG peptide was 5,100 in *D. melanogaster* (Schmidt et al., 1993) and 3,990 in *D. suzukii* (Ohashi et al., 1991), and in the coleopteran *Leptinotarsa decemlineata*, it was 7,971 (Smid et al., 1997). Thus, the MAG peptides in the insects that have been examined have low molecular weights of less than 10,000. The 7,600-mw peptide we isolated also inhibited mating behavior and stimulated oviposition in *Ae.*

aegypti. The amino acid sequence of the active peptide in the MAG secretions of *Ae. aegypti* remains to be identified.

ACKNOWLEDGMENTS

We thank Shuren Wang and Chulhee Kang at the Department of Biochemistry and Biophysics, Washington State University, for their technical assistance with perfusion chromatography. This research was supported by grant IBN-9224357 from the National Science Foundation to M.J.K.

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