ANALYSIS OF SALIVARY GLAND PROTEINS OF THE MOSQUITO *ARMIGERES SUBALBATUS*

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Abstract. Quantitative studies of total salivary gland protein of *Armigeres subalbatus* mosquito revealed that the total salivary gland protein increased dramatically during the five days after emergence as adults. The amount of salivary gland protein of female and male mosquitos at day five after adult emergence were on the average 11.55 and 1.32 μg/pair gland respectively. SDS-PAGE studies showed that salivary gland protein profiles of *Armigeres subalbatus* demonstrated 9 major polypeptide bands of 68, 65, 60, 55, 40, 30, 28, 21, and 15 kDa. The 21 and 65 kDa bands were found only in the distal lateral region of the mosquito salivary gland and were depleted after the female mosquito took a blood meal.

INTRODUCTION

Mosquito-borne diseases still remain a major health problem in both human and veterinary sectors. Diseases transmitted by mosquitos include malaria, dengue hemorrhagic fever, Japanese encephalitis, yellow fever, and filariasis. The pathogens are transmitted to a vertebrate host when the female mosquito takes a blood meal. Many pathogens take up residence in the mosquito salivary glands before being transmitted to a new vertebrate host. In addition, the mosquito saliva may enhance or facilitate infectivity (Ribeiro, 1995; Osorio *et al*, 1996; Edwards *et al*, 1998). Mosquito salivary gland extracts contain α-glucosidases and α-amylases that initiate the digestion of carbohydrates present in dietary carbohydrate sources and other enzymes and peptides involved in blood feeding and ingestion such as anticoagulants, vasodilators, and platelet aggregation inhibitors (Ribeiro and Francischetti, 2003). The saliva also contains molecules that provoke a humoral and cellular immune response in the vertebrate host (Peng *et al*, 1995; Peng and Simons, 1997; Malafront *et al*, 2003).

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The proteins present in the salivary glands of several mosquito species have been investigated (Mellink and van Zenben, 1976; Poehling, 1979; Al-Ahdal *et al*, 1990; Marinotti *et al*, 1990; Andrews *et al*, 1997; Saliman *et al*, 1999; Nascimento *et al*, 2000; Moreira *et al*, 2001; Jariyapan and Harnnoi, 2002; Suwan *et al*, 2002), however, little is known of *Armigeres subalbatus*, a major vector of a heart dog filaria, *Dirofilaria immitis*. In Thailand, *Ar. subalbatus* is the most common early morning and early night biting mosquito and is found throughout the country especially in the rural areas. It feeds on both human and domestic animals (Srinivas *et al*,1994) . The study on salivary gland protein of *Ar. subalbatus* mosquito has never been reported. Therefore, in this study the salivary gland proteins of *Ar. subalbatus* were determined and analysed. Our initial finding on the salivary gland proteins showed significant reduction of some major proteins after blood feeding.

MATERIALS AND METHODS

Maintenance of *Ar. subalbatus* mosquitos

Ar. subalbatus mosquitos were maintained in an insectary of the Department of Medical Sciences, National Institute of Health, Bangkok, Thailand. Conditions were set at 28ºC ± 1ºC at 80% ± 5% relative humidity under 12/12 hours light/dark photo-period. Adults were supplied

with a damp cotton wool pad contained 10% sucrose solution as a carbohydrate source. For blood feeding, female mosquitos were allowed to feed on anesthetized mice for 30 minutes. Groups of mosquitos were reared simultaneously from the same cohort of eggs. Adult mosquitos aged 1 to 5 days after emergence were used.

Salivary gland dissection

Mosquitos were anesthetized on ice and salivary gland dissection was performed as described by Suwan *et al* (2002). Mosquito salivary glands were transferred to a microcentrifuge tube containing a small volume of PBS (phosphate buffer saline solution) and kept at -70ºC until used.

Protein quantification

Amount of total mosquito salivary gland protein was determined using a Bio-Rad Protein Assay (Bio-Rad) following the manufacturer's instruction. Two pairs of female or 10 pairs of male *Ar. subalbatus* salivary glands at day 1, 3, and 5 after emergence were used in this study. Each determination was repeated 3 times.

SDS-PAGE analysis and protein staining

SDS-PAGE was performed according to Laemmli (1970) and the proteins were silver stained using a Silver Stain kit (Bio-Rad) according to the manufacturer's instruction.

RESULTS AND DISCUSSION

The salivary glands of adult *Ar. subalbatus* are paired organs, located in the thorax. The female salivary glands display difference in structure when compared to the male ones. The female gland is composed of two identical lateral lobes and a shorter and wider median lobe (Fig 1). The lateral lobes can be divided into two regions, proximal and distal. The male gland consists of three morphologically homogenous lobes and is approximately one-fifth size of the female (Fig 1). Morphological pattern of *Ar. subalbatus* adult salivary glands followed the same pattern as described for *Ae. aegypti* (Mellink *et al*, 1976; Poehling, 1979), *Ae. albopictus* (Marinotti *et al*, 1996), *Ae togoi* (Jariyapan and Harnnoi, 2002), *Cx. pipiens* and *Ae. caspius* (Saliman *et al*, 1999).

Total protein contents of male and female mosquito salivary glands were determined. Table 1 demonstrates the average amount of total salivary gland protein content in male and female mosquitos at various times after emergence. Total salivary gland protein content of male mosquito on day 1 after emergence was 0.44 ± 0.10 μg/gland pair and increased to 1.32 ± 0.14 μg/gland pair at day 5. In females, total salivary gland protein content of mosquito at day 1 after emergence was 1.85 ± 0.53 μg/gland pair and increased dramatically to 11.55 ± 1.71 μg/gland pair at day 5. Comparison of protein content between male and female mosquitos at day 5 showed that a pair of male glands contained approximately 10% of that found in female ones. Similar results were found in *Cx. pipiens* (Saliman *et al,*1999) and *Ae. togoi* (Jariyapan and Harnnoi, 2002).

Fig 1–Salivary glands of *Armigeres subalbatus* mosquito. A: a male salivary gland; B: female salivary gland, M: median lobe; L: lateral lobe; PL: proximal region of lateral lobe; DL: distal region of lateral lobe. The photographs were taken using an Olympus (AH) microscope at 100x magnification.

SDS-PAGE analysis of the proteins present in the salivary glands of male and female mos-

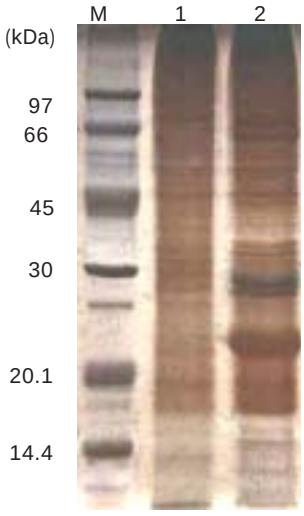

Fig 2–Protein electrophoresis pattern of salivary glands of adult male and female *Armigeres subalbatus* at day 5 after adult emergence. Ten pairs of sugar fed female salivary glands (lane 2) and 15 pairs of male salivary glands (lane 1) were submitted to SDS-PAGE in a 12% polyacrylamide gel followed by silver staining. Molecular mass markers (M) are in kilodalton (kDa).

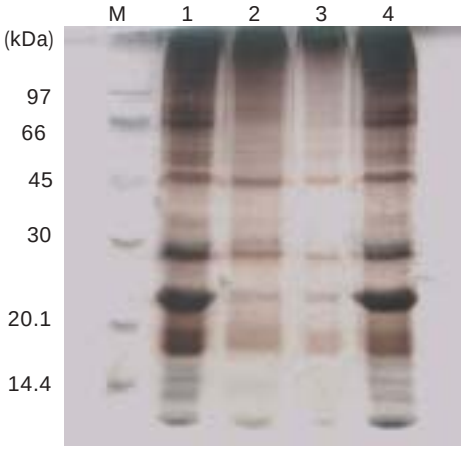

Fig 3–Electrophoretic profile of polypeptides from female *Armigeres subalbatus* mosquitos salivary gland lobes. Proteins were separated on a 12% SDS-PAGE gel and silver stained. Lane 1,ten whole female salivary glands; lane 2, twenty proximal lateral lobes; lane 3, ten median lobes; lane 4, twenty distal lateral lobes. M: molecular weights markers of sizes (kDa) indicated on the left side of the picture.

quitos at day 5 of emergence was performed. The analysis of both female and male salivary gland proteins revealed the presence of at least 9 major and several minor protein bands (Fig

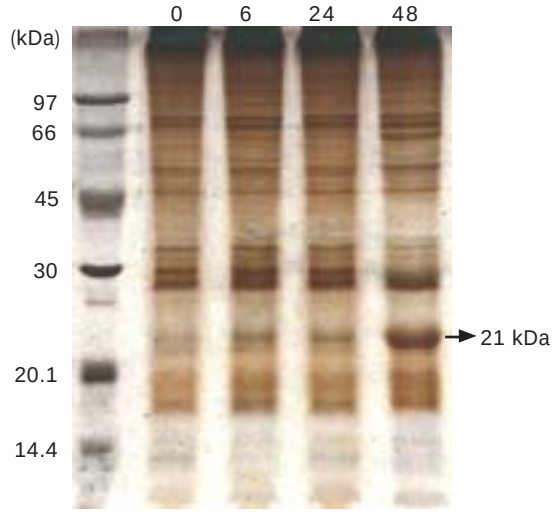

Fig 4–Protein electrophoretic profile of salivary glands of blood fed *Armigeres subalbatus* mosquitos. Ten pairs of salivary glands were dissected from blood fed mosquitos at 0, 6, 24 and 48 hours after a blood meal and submitted on 12% SDS-PAGE and silver stained. M: molecular weight markers of sizes (kDa) indicated on the left side of the picture. Number at the top indicate hours after a blood meal.

Table1
Total salivary gland protein contents of male and female *Armigeres subalbatus* mosquitos after the adult emergence.

	Post emergence (day)					
	Male			Female		
	1	3	5	1	3	5
Protein content (μg/gland pair)[A]						
	0.44 ± 0.10	0.49 ± 0.14	1.32 ± 0.14	1.85 ± 0.53	6.53 ± 0.78	11.55 ± 1.71

A = mean ± SD; n = 10

2). Protein bands with estimated molecular masses of 68, 60, 55, 40, 30, 28 and 15 kDa were found in salivary glands of both sexes whereas the 65 and 21 kDa protein bands were observed only in females.

The different morphological regions of the female salivary glands displayed distinct protein profiles (Fig 3). The 65 and 21 kDa proteins appeared predominantly in the distal-lateral lobe. The protein profile of the female proximal-lateral lobe was similar to that of the male salivary

gland. The protein profiles at 0, 6, 24, and 48 hours after a blood meal was also analyzed (Fig 4). Immediately after blood feeding, the 65 and 21 kDa protein bands were barely detected, but both proteins started to appear gradually 6 hours later and returned to the unfed level in 48 hours. Further investigations in molecular, biochemical, and immunological aspects of *Ar. subalbatus* salivary glands will provide information for better understanding of the role of mosquito salivary gland proteins in blood feeding and disease transmission.

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