Decrease of Mosquito Salivary Gland Proteins after a Blood Meal: An Implication for Pathogenesis of Mosquito Bite Allergy

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Salivary gland protein profiles of Aedes aegypti *(L.) and* Culex quinquefasciatus *(Say) pre- and post-blood feeding were analyzed. SDS-PAGE studies before blood feeding of* Ae. aegypti *demonstrated 8 major polypeptide bands of 20, 35, 37, 42, 45, 47, 70 kDa and a high molecular weight band > 118 kDa, whereas those of* Cx. quinquefasciatus *demonstrated 9 major polypeptide bands of 20, 26, 36, 38, 45, 47, 49 kDa and 2 high molecular weight bands > 118 kDa. After a blood feeding, salivary gland polypeptides of Ae. aegypti at 35, 37, 45, 47, 70 kDa and high molecular weight band > 118 kDa were depleted, while the polypeptide bands of 20, 26, 36, 38 kDa were depleted in* Cx. quinquefasciatus*. The presented study suggests that these major polypeptides were introduced into vertebrate hosts when a mosquito took a blood meal. Further investigation in molecular, biochemical and immunological aspects of these salivary gland polypeptides may provide information for better understanding in the role of these proteins in mosquito bite allergy.*

Keywords: *Mosquito bite allergy, Aedes aegypti, Culex quinquefasciatus, Mosquito salivary gland protein*

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Dermal allergy to mosquito bites is a common problem worldwide. Although in most cases of mosquito bites elicit mild symptoms such as cutaneous reactions, systemic reactions including generalized urticaria and angioedema, rhinitis, conjunctivitis, asthma have been documented[1-3]. Anaphylactic shock following mosquito bites also has been reported[4]. These reactions are caused by proteins in the mosquito saliva and involved in IgE, IgG1 and IgG4 responses and lymphocyte proliferation[5,6].

Mosquito saliva contains 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[7,8]. The saliva also contains molecules that provoke a humoral and cellular immune response in the vertebrate host[9-11]. Although salivary glands of several mosquito species have been investigated[7,12-20], changes of salivary gland protein post blood feeing using SDS-PAGE was demonstrated only in Armigeres (Ar.) subalbatus (Coquillett) mosquito[20].

In Thailand, Aedes (Ae.) aegypti (L.) and Culex (Cx.) quinquefasciatus (Say) mosquitoes are the most important mosquito species distributed throughout the country. Ae. aegypti is the most important endophagic, daylight-bite mosquito and plays a major role of dengue virus transmission. Cx. quinquefasciatus is exophagic, night-bite mosquito found mainly in urbanized areas. Mosquito bite allergy is a common problem found in clinical practice especially in children. Despite this, only a few reports in which modern laboratory techniques have been applied to the study of mosquito allergy in Thailand[21]. In the present study the authors would like to determine the major polypeptides which were related to blood feeding of Ae. aegypti and Cx. quinquefasciatus by SDS-PAGE. This would provide crucial information for further investigation in mosquito bite allergy.

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Material and Method

Mosquito rearing

Ae. aegypti and Cx. quinquefasciatus mosquitoes were raised in an insectary at the Experimental Animal Unit, Faculty of Medicine, Chulalongkorn University. Briefly, after the emergence as adults, the mosquitoes were reared in insectariums at 28°C ± 1°C, 80% ± 5% relative humidity under 12/12 hours light/dark photo-period. Adults were supplied with a damp cotton wool pad which contained 10% sucrose solution as a carbohydrate source until used.

Mosquito blood feeding

Female mosquitoes were allowed to feed on anaesthetized mice for 30 minutes. Groups of mosquitoes were reared simultaneously from the same cohort of eggs. Adult mosquitoes aged 4 to 5 days after emergence were used.

Mosquito salivary gland extraction

Mosquito salivary gland extracts were prepared from 5 days old female mosquitoes. Mosquitoes were anaesthetized on ice and salivary gland dissection was performed as in the method described by Suwan et al. (2002)[18]. Mosquito salivary glands were then transferred to a microcentrifuge tube containing a small volume of PBS (phosphate buffer saline solution) and kept at -70°C until used.

SDS-PAGE Analysis

SDS-PAGE was performed according to Laemmli (1970)[22] and the proteins were stained using a Coomassie Brilliant Blue (PhastGel™ Blue R) according to the manufacturer's instruction. Twenty pairs of mosquito salivary glands were used for each sample, and each experiment was repeated three times.

Results

Morphology of mosquito salivary glands

The salivary glands of female Ae. aegypti and Cx. quinquefasciatus are paired organs, located in the thorax. The gland is composed of two identical lateral lobes and a shorter and wider median lobe. The lateral lobes could be further divided into two regions, proximal and distal. Salivary glands of these two mosquito species are undistinguishable morphologically (data not shown).

SDS-PAGE Analysis

SDS-PAGE analysis of salivary gland proteins of female Ae. aegypti mosquito pre-blood feeding demonstrated 8 major polypeptide bands of 20, 35, 37, 42, 45, 47, 70 kDa and a high molecular weight band >118 kDa. After a blood meal, the depletion of major peptide bands of 35, 37, 45, 47, 70 kDa and high molecular weight band >118 kDa was observed (Fig. 1).

Study in Cx. quinquefasciatus found 9 major polypeptide bands of 20, 26, 36, 38, 45, 47, 49 kDa and 2 high molecular weight bands >118 kDa, the polypeptide bands of 20,26, 36 and 38 kDa were depleted after a blood feeding (Fig. 2).

Discussion

Morphology of Ae. aegypti and Cx. quinquefasciatus from the present study is similar to the pattern described for Ae. aegypti[13,14], Ae. albopictus (Skuse)[23], Ae togoi (Theobald)[19], Cx. pipiens (L.), Ae. caspius (Pallas)[15], and Ar. subalbatus[20]. The female gland is composed of two identical lateral lobes and a shorter and wider median lobe. The lateral lobes could be further divided into two regions, proximal and distal.

The salivary gland protein profile of Ae. aegypti and Cx. quinquefasciatus mosquito showed a

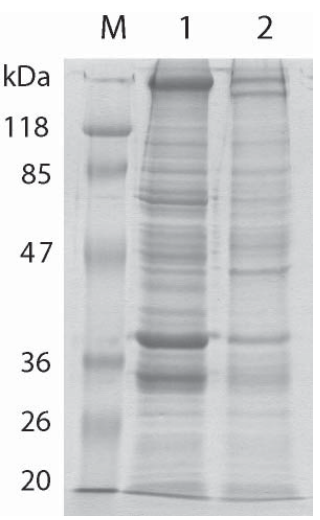

Fig. 1 Protein electrophoretic profile of salivary glands of *Aedes aegypti* mosquitoes. Proteins were separated on a 12% SDS-PAGE gel and Commasse Brilliant Blue stained. Lane 1, twenty pairs of salivary glands of female mosquitoes at day 5 after emergence (sugar feeding); Lane 2, twenty pairs of salivary glands of female mosquitoes dissected immediately after a blood meal; M: Molecular weights markers of sizes (kDa) indicated on the left side of the picture

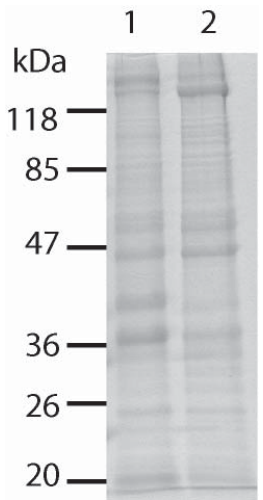

Fig. 2 Protein electrophoretic profile of salivary glands of *Culex quinquefasciatus* mosquitoes. Proteins were separated on a 12% SDS-PAGE gel and Commasse Brilliant Blue stained. Lane 1, twenty pairs of salivary glands of female mosquitoes at day 5 after emergence (sugar feeding); Lane 2, twenty pairs of salivary glands of female mosquitoes dissected immediately after a blood meal; Molecular weights markers of sizes (kDa) indicated on the left side of the picture

different pattern. The different protein profiles are found not only in different species but also in the same mosquito species. Study by Moreria et al. demonstrated that Anopheles darlingi (Root) mosquito collected from different geographical regions of Brazil showed some differences in pattern of salivary gland protein profile[17]. In the present study the authors demonstrated the salivary gland protein profile of Ae. aegypti and Cx. quinquefasciatus which originally were collected from Bangkok and maintained at the insectary of the National Institute of Health, Department of Medical Sciences, Nonthaburi, Thailand.

Decreasing of major peptide bands of 35, 37, 45, 47, 70 kDa and a high molecular weight band >118 kDa in Ae. aegypti and 20, 26, 36 and 38 kDa in Cx. quinquefasciatus indicate that these polypeptide proteins were released to vertebrate hosts while female mosquitoes took a blood meal. Therefore, these salivary gland proteins may cause mosquito bite allergy in human. Hudson et al. (1960) demonstrated that mosquito saliva was a source of antigens which produced typical bite reaction in man[24] and Peng et al. (1996) showed that recombinant 37 kDa protein in Ae. aegypti was shared by all five Aedes species and also Cx. quinquefasciatus mosquito[5]. In the present study the authors also found the 37 kDa salivary gland protein in Aedes aegypti mosquito and this protein was depleted after blood feeding.

Nasciomento et al. (2000) and Malafronte et al. (2003) demonstrated that salivary gland proteins of Cx. quinquefasciatus mosquito had 2 major polypeptide bands of 28.3 and 35.7 kDa, which induced immune response in mice[11,16]. In the present study the authors also showed 36 kDa polypeptide band that related to blood feeding. But were unable to demonstrate depletion of the 28.3 kDa polypeptide protein in the present study.

At present, laboratory diagnosis of mosquito bite allergy using the commercial mosquito extracts prepared from whole mosquitoes are not standardized for diagnosis of mosquito allergy[25]. In order to improve the precision of diagnosis of mosquito allergy, purified mosquito saliva should be developed. The present study provides data of salivary gland proteins, which related to blood feeding. Therefore, these proteins may be related to mosquito bite allergy. Further study of these purified or recombinant salivary gland proteins would help physicians to diagnose mosquito bite allergy more accurately.

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References

1. Frazier CA. Biting insects. Arch Dermatol 1973; 107: 400-2.
2. Gluck JC, Pacin MP. Asthma from mosquito bites: a case report. Ann Allergy 1968; 8: 47-57.
3. Walker GB, Harrison PV. Seasonal bullous eruption due to mosquitoes. Clin Exp Dermatol 1985; 10: 127-32.
4. McCormack DR, Salata KF, Hershey JN, Carpenter GB, Engler RJ. Mosquito bite anaphylaxis: immunotherapy with whole body extracts. Ann Allergy Asthma Immunol 1995; 74: 39-44.
5. Peng Z, Simons FER. Comparison of proteins, IgE and IgG binding antigens, and skin reactivity in commercial and laboratory-made mosquito extracts. Ann Allergy Asthma Immunol 1996; 77: 371-76.
6. Peng Z, Simons FER. Mosquito Allergy: Immune

mechanisms and recombinant salivary allergens. Int Arch Allergy Immunol 2004; 133: 198-209.
7. Marinotti O, James AA, Ribeiro JMC. Diet and salivation in female Aedes aegypti mosquitoes. J Insect Physiol 1990; 36: 545-8.
8. Ribeiro JM, Francischetti IM. Role of arthropod saliva in blood feeding: sialome and post-sialome perspectives. Annu Rev Entomol. 2003; 48: 73-88.
9. Peng Z, Yang M, Simons FER. Measurement of mosquito Aedes vexans salivary gland-specific IgG antibodies and the distribution of the antibodies in human sera. Ann Allergy Asthma Immunol 1995; 74: 259-64.
10. Peng Z, Simons FER. Cross-reactivity of skin and serum specific IgE responses and allergen analysis for three mosquito species with world-wide distribution. J Allergy Clin Immunol 1997; 100: 192-8.
11. Malafronte R dos S, Calvo E, James AA, Marinotti O. The major salivary gland antigens of Culex quinquefasciatus are D7-related proteins 2003. Insect Biochem Mol Biol 2003 ; 33: 63-71.
12. Al–Ahdal MN, Al–Hussain K, Thorogood RJ, Rrilly HC, Wilson JD. Protein constituents of mosquito saliva: studies on Culex molestus. J Trop Med Hyg 1990; 93: 98-105.
13. Mellink JJ, van Zeben MS. Age related difference of saliva composition in Aedes aegypti. Mosq News 1976; 36: 247-50.
14. Poehling HM. Distribution of specific proteins in the salivary gland lobes of Culicidae and their relation to age and blood sucking. J Insect Physiol 1979; 25: 3-8.
15. Soliman MA, Abdel-Hamid ME, Mansour MM, Seif AM, Kamel KI, El Hannshary EM. Total salivary gland proteins of female Culex pipiens and Aedes caspius (Diptera:Culicidae) and their fractionation during adult development and after blood sucking. J Egyp Soc Parasitol 1999; 29: 619-34.
16. Nascimento EP, Malafronte R dos S, Marinotti O. Salivary gland proteins of the mosquito Culex quinquefasciatus. Arch Insect Biochem Physiol 2000; 43: 9-15.
17. Moreira CK, Marrelli MT, Lima SP, Marinotti O. Analysis of salivary gland proteins of the mosquito Anopheles darlingi (Diptera: Culicidae). J Med Entomol 2001; 38: 763-7.
18. Suwan N, Wilkinson MC, Crampton JM, Bates PA. Expression of D7 and D7 related proteins in the salivary glands of the human malaria mosquito, Anopheles stephensi. Insect Mol Biol 2002; 11: 223-32.
19. Jariyapan N, Harnnoi T. Preliminary study of salivary gland proteins of the mosquito Aedes togoi (Theobald). Chiang Mai Med Bull 2002; 41: 21-8.
20. Siriyasatien P, Tangthongchaiwiriya K, Jariyapan N, Kaewsaitiam S, Poovorwan Y, Thavara U. Analysis of mosquito salivary gland proteins of the mosquito Armigeres subalbatus. Southeast Asian J Trop Med Public Health 2005; 36: 64-7.
21. Chumdermpadetsuk S, Lekagul P, Boonyaratavej K, Pongprasit P. Skin Sensitivity to mosquito antigen. J Med Assoc Thai 1981; 64: 382-5.
22. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680-5.
23. Marinotti O, de Brito M Moreira CK. Apyrase and α-glucosidase in the salivary glands of Aedes albopictus. Comp Biochem Physiol 1996; 113B: 675-9.
24. Hudson A, Bowman L, Orr CWM. Effects of absence of saliva on blood feeding by mosquitoes. Science 1960; 131: 1730-1.
25. Peng Z, Yang M, Simons FER. Immunologic mechanisms in mosquito allergy: correlation of skin reactions with specific IgE and IgG antibodies and lymphocyte proliferation response to mosquito antigens. Ann Allergy Asthma Immunol 1996; 77: 238-44.

การลดลงของโปรตีนในต่อมน้ำลายยุงหลังการดูดเลือด: พยาธิกำเนิดของการแพ้ยุงกัด

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การวิเคราะห์โปรตีนในต่อมน้ำลายยุงลายบ้าน (Aedes aegypti) และ ยุงรำคาญ (Culex quinquefasciatus) โดยวิธี SDS-PAGE ทั้งก่อนและหลังให้ยุงดูดกินเลือด พบว่าโปรตีนหลักในต่อมน้ำลายยุงลายบ้านมีอยู่ 8 ขนาด ได้แก่ โปรตีนขนาด 20, 35, 37, 42, 45, 47, 70 kDa และโปรตีนที่มีน้ำหนักโมเลกุลมากกว่า 118 kDa อีก 1 แถบ สำหรับโปรตีน ในต่อมน้ำลายยุงรำคาญมีอยู่ 9 ขนาด ได้แก่ โปรตีนขนาด 20, 26, 36, 38, 45, 47, 49 kDa และโปรตีนที่มีน้ำหนักโมเลกุลมากกว่า 118 kDa อีก 2 แถบ โปรตีนในต่อมน้ำลายยุงทั้ง 2 ชนิดมีการเปลี่ยนแปลงภายหลังยุงดูดกินเลือด โดยพบว่าโปรตีนในต่อมน้ำลายขนาด 35, 37, 45, 47, 70 kDa และ โปรตีนที่มีน้ำหนักโมเลกุลมากกว่า 118 kDa ของยุงลายบ้านมีปริมาณลดลง และโปรตีนในต่อมน้ำลายขนาด 20, 26, 36 และ 38 kDa ของยุงรำคาญมีปริมาณลดลง ผลการศึกษาแสดงให้เห็นว่ามีโปรตีนในต่อมน้ำลายยุงถูกปล่อยเข้าสู่โฮสท์ขณะยุงดูดกินเลือด ซึ่งสามารถใช้เป็นแนวทางในการศึกษาคุณสมบัติทางอณูชีววิทยา ชีวเคมีและภูมิคุ้มกันวิทยาของโปรตีนเหล่านี้ และจะช่วยทำให้เกิดความเข้าใจบทบาทของโปรตีนเหล่านี้ในพยาธิกำเนิดของการแพ้ยุงกัดมากยิ่งขึ้น