Protein Profiling of Accessory Glands and Duplex of Male *Helicoverpa armigera*

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Abstract: Protein profile of virgin male accessory glands and duplex (MAG-duplex) extract constructed, from 0-36 hours of eclosion at an interval of 6 hours, on Tricine–SDS-PAGE showed the appearance of <7kDa band only after 12 hrs of eclosion and the concentration gradually increases with age of the moth reaching maximum, probably a critical concentrate on, around 24 hrs and that is the time when males are ready for mating. Probably the peptide/peptides of <7kDa band may responsible for the behavioral changes shown by the mated females.

Keywords: Helicoverpa armigera, male accessory gland, peptide, behavioral regulation

1. Introduction

Studies over the past six decades have shown that in many insect species, mating profoundly influence the female to remodel her reproductive behavior and physiology as a consequence of altered gene expression in turn influenced by male origin factors. These changes trigger responses in several processes that lead to the production of progeny. In many insect females, mating elicits a series of well-defined behavioral changes [1]. Among the most conspicuous are (1) an increase in egg-laying rate and (2) the reduction of receptivity [2-5], the earlier, to the advantage of female and the latter to the advantage of male. These changes in female reproductive behavior are induced by the substances synthesized in the tissues of male reproductive system and transferred as part of the seminal fluid to the female during copulation [6-8]. Numerous studies have demonstrated that the male accessory glands are the key secretory structures contributing to the seminal fluid that play an essential role in insect reproduction. Their main function being the production of the spermatophore for sperm transfer from male to female, changes in the behavior of females is also attributed to the peptides/proteins present in their secretion.

2. Materials and Methods

2.1. Insects and culture

A starter culture, neonates of *H. armigera* (NBAII-MP-NOC-01) was procured from NBAIR, Bengaluru, were reared on modified semi synthetic chickpea diet developed by Shobha et al [9]. The 3rd instar larvae were maintained individually in a plastic cup at 25 °C, 65±5% RH and 16L/8D conditions in a B.O.D incubator. Pupae were collected and sexed according to the characteristics of their exterior paramera. The male and female pupae were kept separately in plastic boxes until adult emergence to ensure virginity and age.

2.2. Preparation of MAG-duplex extract for Tricine–SDS-PAGE

MAG-duplex tissue from the virgin males emerged at six hour intervals (0-36hrs) was pooled in vials placed on ice. The tissue was homogenized using Milli-Q water (20 µl/moth) with micro pestle (Tarson) and centrifuged at 12000 g for 15 min at 4 °C. The supernatant was collected carefully without fat and stored at -40 °C.

2.3. Tricine–SDS-PAGE

Proteins were separated by one-dimensional Tricine-SDS-PAGE with Tricine-Tris buffer systems.
Resolving (16%), spacer (10%) and stacking (4%) gels were prepared [10]. The 20 µl of samples collected at 6 hour interval were mixed with 4X sample buffer (12% SDS, 30% glycerol, 0.05% coomassie blue G-250, 150 mM Tris/HCl, pH 7.0) and were loaded on the gel. The proteins/peptides bands were visualized by staining the gel with coomassie blue R-250 (SRL) after placing the gel in fixing solution (50% methanol, 10% acetic acid, 100 mM ammonium acetate). The molecular weights of the bands were determined by comparison with the electrophoretic mobilities of a series of low-range protein standards (SRL BioLit).

3. Result

Protein profile of virgin MAG-duplex from 0-36 hrs after eclosion, at an interval of 6 hrs, showed the peptide band of <7kDa and was appeared only after 12 hrs of eclosion. The concentration gradually increased with the age of the moth and reached maximum at around 24 hrs. The male moths start to mate only on the second day scotophase i.e. after 24 hrs of emergence though in small percentage.

4. Discussion

The study was carried out to know the time of release peptide/peptides responsible for regulating the female reproductive behaviors. The peptide/peptides band was found to be <7kDa and appear around 12h after emergence of the moth and reaches critical concentration at 24h, supporting the study carried out by Hou [11]. According to him, generally, male H. armigera become sexually mature by 24hrs after emergence and fully mature by 48h post-emergence. However in various insects, the degree of reproductive development and activity at adult emergence varies greatly [12]. In some insects such as the silkmoth, Bombyx mori, or the gypsy moth, Lymantria dispar gametogenesis is complete at the time of eclosion, and adults of these species do not feed and are ready for immediate mating and oviposition, with no need for hormonal regulation.

This age-based behavioural plasticity depends on the biosynthesis activity of juvenile hormone, which controls the sensitivity of neurons in the primary olfactory centre, the antennal lobe [13, 14]. The previous studies have revealed that the sensitivity of the peripheral pheromone detection system on the antennae of A. Ipsilon males is age and JH-independent [15]. Age and hormone dependent changes in olfactory-guided behaviour have been found in a few insect species. Male moths change their responsiveness to sex pheromones with age and endocrine situation in migratory species [15, 16], whereas males in non-migratory species respond to the female sex pheromone directly after hatching [17].

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6. References

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