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Sajitha N and Kavitha KP

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RESEARCH ARTICLE

STUDIES ON THE SILK PROTEINS OF THE SILK GLANDS OF VARIOUS DEVELOPMENTAL STAGES OF THE MANGO LEAF WEBBER, ORTHAGA EXVINACEA HAMPSON (LEPIDOPTERA:PYRALIDAE)

Sajitha N * and Kavitha KP

Department of Zoology, Govt. College Chittur, Palakkad-678104

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ABSTRACT

The protein present in the silk glands of different larval instars and pre-pupal stages of *O. exvinacea* were estimated. The result showed that the amount of protein present in the silk glands was found to increase from the Vth to VIIth instars and then decreasing towards pre-pupal stage. The molecular weight of individual proteins present in the silk glands of different larval instars was determined using SDS-PAGE. There were seven visible bands present in 10% SDS-PAGE. The molecular weights of individual proteins present in the silk glands of different larval instars were in between 127 to 29 kDa. These proteins were sometimes the glycoproteins that were essential for the secretion of the web or the glue protein like sericin.

INTRODUCTION

Silk is produced by a number of organisms, of which silk producing lepidopteran insects became the major focus of this millennia. Moreover, lepidopterans were used as an experimental model for research in the physiological and developmental processes. The most common mulberry silk is produced by *Bombyx mori*. In addition to it, tasar, eri and muga silk produced by other lepidopterans were a major contribution to Indian textile industry.

Silk proteins are synthesized in by different types of glands in insects (Sehnal and Akai, 1990), which include collateral sex glands, malpighian tubules, gut, labial glands different types of dermal glands, etc. In Lepidoptera, modified, paired, long labial glands of larvae were found to be evolved to spin silk. These are ectodermal in origin (Rudall and Kenchington, 1971), anatomically and physiologically distinguished into anterior, middle and posterior regions in *B. mori* (Akai, 1983). A few species of caterpillars spin silk throughout there larval phase and others secrete silk as a small girdle to hold up the larvae during molting. Metamorphosis is the decisive phenomenon in the development of silkworm *B.mori* (Chen, 1971). Durable cocoon was spun by certain species of lepidopterans during there pupal period. Certain lepidoteran species spin durable cocoon as the larvae pupate. Sericin and fibroin are the two major proteins present in lepidopteran silk (Altman *et al.*, 2003). Sericin covers the surface of fibroin in the cocoon of *B. mori*. Sericin is produced by the middle portion of silk gland and fibroin by the posterior portion. The anterior portion of silk gland serves as an outlet terminating with spinneret, from which the silk fibres start spinning (Sehnal and Akai, 1990).

The demand for silk and silk protein has been increasing day by day. In this situation, the investigations aimed at determining, the structure of silk glands, the total protein present in it and its molecular weight in the different developmental stages of the mango leaf webber, *Orthaga exvinacea* Hampson have influential role to play.

MATERIALS AND METHODS

Experimental insect

The study was conducted on *Orthaga exvinacea* Hampson, which is a serious caterpillar pest of mango trees infesting during the period of December to April of the year.

Larvae were collected from their natural habitat, transferred to plastic jars kept in the insectary and reared by feeding mango

*Corresponding author: **Sajitha N**
Department of Zoology, Govt. College Chittur, Palakkad-678104

leaves. The plastic jars were cleaned in alternate days. The colony was maintained at 27± 2^{0}C and 70%-80% RH.

Collection of silk glands from different larval instars

Freshly moulted larvae of different instars were selected, cut mid dorsally and the digestive system was removed. Pairs of silk glands each (30 nos) were taken from all instars and washed in insect Ringer solution. They were kept in eppendorf tube containing ice-cold insect ringer solution and was stored at -20^{0}C. Frozen silk glands were thawed and homogenized in 1% SDS solution. The homogenate was subjected to centrifugation at (8000 rpm) at 4^{0}C for 10 min. The supernatant was collected for quantification of proteins. The amount of protein was measured by modified Lowry method (1951) using Spectrophotometer (Shimadzu). The data were subjected to statistical analysis. The samples of silk glands homogenate were subjected to SDS PAGE analysis.

RESULTS

Table 1 Total protein present in the silk glands of different developmental stages of *O. exvinacea*

SL. No.	Different stages	Amount of protein(µg/pair of gland)
1	5th	46.01 ± 0.10
2	6th	52.13 ± 0.12
3	7th	61.56 ± 0.13
4	Pre-pupal stage	46.68 ± 0.12

Values are expressed as means ± SEMs, n=7

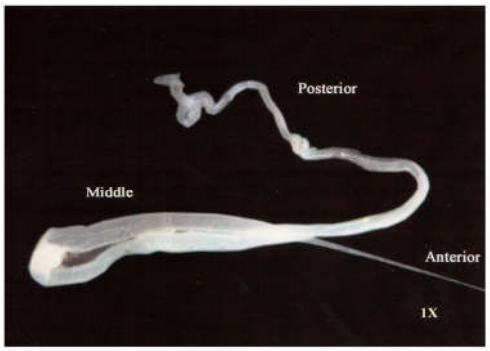

Fig.1. Silk gland of *O.exvinacea*

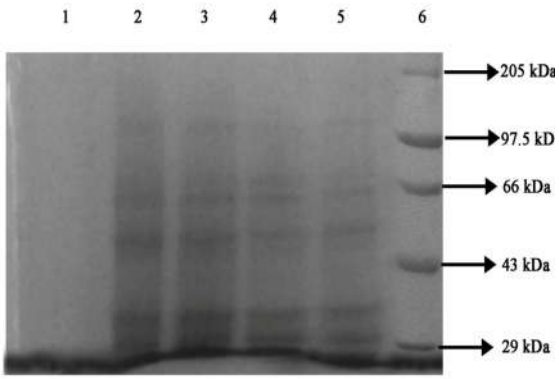

Fig.2. SDS-PAGE analysis of proteins present in the silk glands of different developmental stages of *O.exvinacea* stained with Coomassie brilliant blue R-250.

Lane 2 Fifth instar, Lane 3 Sixth instar, Lane 4 Seventh instar, Lane 5 Pre-pupal stage and Lane 6 Standard protein marker.

Structure of silk gland in O. exvinacea

The silk gland of *O. exvinacea* larva was seen as a paired structure, one on either side of the alimentary canal (Fig. 1). The glands are very delicate and almost transparent. It could be clearly distinguished into three distinct regions. Anterior region is thread like, very thin and attached to the spinneret situated behind the mandible. Middle region is slightly opaque, bulky and 'S' shaped. Posterior region is thinner compared to the middle and it is uniform in diameter. The total length of the silk glands in the final larval instar ranges in between 20.5 mm to 30.2 mm.

Total protein present in the silk glands of different stages of development of O. exvinacea

The content of protein present in the silk glands of different developing stages of *O. exvinacea* were shown in Table 1. In the Vth instar larva, the protein content was 46.01±0.10 µg/pair of silk glands. The amount increased to 52.13±0.12 µg/pair of silk glands in the VIth instar. Maximum protein content, 61.56 ±0.13 µg/ pair of silk gland was estimated in the VIIth instar. However, during pre-pupal stage, the protein content showed a decline. The amount was 46.68 ± 0.12 µg/pair of silk glands.

Protein maps of the silk protein present in the silk glands of different developmental stages of O. exvinacea using SDS-PAGE

The proteins isolated from the silk glands of different developmental stages were separated on SDS-PAGE according to the method described by laemmli (1970). Protein samples were loaded in each well. The approximate molecular weight of proteins were determined manually by plotting the log of molecular weight of the standard against its relative mobility (Fig. 2). This MWs were also confirmed in a gel documentation system using quantity One Program. Most of the bands show high intensites in the lane 3 and 4, which represent VIth and VIIth larval instars. In pre-pupal stage (Lane 5, Fig. 2) all bands are present but the intensities of the bands are low. The protein bands separated on the gel were estimated to have approximate molecular weights of 127 kDa, 75 kDa, 66 kDa, 52 kDa, 45 kDa, 36 kDa and 29 kDa.

DISCUSSION

The present work is an investigation on the silk producing glands of *O. exvinacea* and the proteins of silk glands and the silk. The silk gland of *O. exvinacea* is divided into anterior, middle and posterior portions (Fig. 1). This is similar to the structure of silk glands in the case of silkworm, *B. mori*, where also the silk glands is divided into three regions (Suzuki, 1977). In the present study the total protein content of silk glands showed gradual increase from Vth to VIIth larval instars and a decrease in the pre-pupal stage (Table 1). In *B. mori* similar results were reported by Sarangi (1985). Total protein content in the middle portion of the silk glands increased gradually upto the spinning stage but decreased sharply in the case of posterior portion of the gland. Separate analysis of protein in the middle and posterior portions of silk glands were difficult

because they were too small in size. In pupal and adult stage silk glands has no significance, as web formation takes place only in the larval stage. In *B. mori*, cytolysis of silk glands have been reported during metamorphosis from larva to pupa (Matsuura *et al.,* 1968). In *Orthaga,* silk glands are absent in the pupal stage and this may be due to the cytolysis of silk glands in the insect as in the case of *B. mori.*

Scriber and Slanky (1981) analysed the pattern of food consumption and utilization in *B. mori* larvae, and the maximum level of protein observed in the pre-moulting stage was correlated to the maximum feeding period of the instars. *B.* mori consume more than 70% of the total food during the final instar and this period is characterized by active growth and specifically in silk gland weight (Walbauer, 1986). The protein pattern in the silk glands of different instars of *O. exvinacea* agree with the pattern found in *B. mori*. This also points to the possibility of common pattern of feeding and silken web production in insects which produce silk.

The major silk proteins in *B. mori* are fibroin and sericin. The fibroin consist of one H-chain of 350 kDa (Oyama *et al*., 1984), L-chain of 25 kDa and two P25 proteins of 27 and 29 kDa (Tanaka *et al.,* 1993). Sericin is a globular and water soluble protein with molecular weight ranges from about 10 kDa to 300 kDa (Wei *et al*., 2005). Analysis of proteins present in silk glands in *Orthaga* shows that it is having a molecular weight in between 29 kDa to 127 kDa (Fig. 2). The protein present in the silk glands of this insect may be of low molecular weight glycoprotein associated with fibroin or glue protein like sericin. The web formation was more in V^{th} and VI^{th} instars than VII^{th} larval instar, the intensity of protein bands with molecular weights 66, 52, 45 kDa were higher in V^{th} and VI^{th} stage (Fig. 2). Glue protein like sericin is essential for webbing of leaves.

Interest in silk has increased with a near explosion of biomaterial research and applications. Other natural silk resources are spiders, honey bees, hornets, solitary wasps etc. *Orthaga* which produce web through out the larval stages, is a feature that distinguishes *Orthaga* from *B. mori*, where *Bombyx* produces cocoon only in the last larval instar. Our preliminary investigations showed that we need a full exploitation of silk proteins using advanced technologies. Like spider silk, this pyralid silk and silk proteins may have some applications not only in the field of material science and engineering but also in green chemistry and biomedicine.

CONCLUSION

The industrial demand for silk is increasing, not only in clothing but cosmetic industry also have high demand for silk protein i.e sericin and fibroin. Sericin alone or in combination with fibroin has been used in skin, hair and nail cosmetics. It is found that when sericin used in the form of ointment, lotion and cream shows increased skin elasticity, antiwrinkle and antiaging effects. The major constraints faced by silk industry is about production.

So to overcome this huge demand there is a need of a potent alternative. This information suggest that the use of mango webber silk as good substitute in various cosmetic and biotechnological applications.

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