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PROTEOMIC ANALYSIS AND CHARACTERIZATION OF SILK PROTEINS IN SILKWORM (*BOMBYX MORI* L.)

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ABSTRACT

Of the various insect species silk producing insects which includes *Bombyx mori* and *Antherae mylitta* are very famous as these are highly beneficial to the mankind. They produce silk which is worldwide recognized as the Queen of fibres. The demand of silk is increasing in the world day by day and therefore many scientists are involved to increase the production and productivity of silk by using latest technologies. . Natural fibres have an edge over artificial fibres and silk excels all the fibres for a number of inherent characteristics such as lusture, softness, elegance, versatility, wearability, yarn strength etc. Silk fibre is constituted by two important proteins, fibroin (73.5 %) and sericin (22.28 %).These proteins are the complex organic nitrogenous substances. Proteins occupy a central position in the architecture as well as functioning of living matter. Proteomics is a newly emerging field of life science research, using high throughput technologies to display, identify or characterize all the proteins in a given cell, tissue or organism. Three approaches are mainly used in proteomics viz., Gel-based proteomics, mass spectrometry driven proteomics and protein arrays. Gel based proteomics is the older approach to screen the protein expression at large scale and it includes (IFF) First dimensional isoelectric focursing (Bravo et al., 1981) and Second dimensional SDS-PAGE (Gorg et al., 1988) . The present paper overviews some important studies carried out on silk proteins viz. sericin and fibroin of silkworm, *Bombyx mori* L.

INTRODUCTION

The physiology of an insect is greatly altered during development from embryo to adult. For example, the complete metamorphosis exhibited by an insect such as the silkworm involves drastic changes. The growth of silk gland in silkworm is of paramount importance to the sericultural industry as it is responsible for the synthesis of silk proteins, the basic raw materials of the silk cocoon. Prominently, the silk gland grows during the fourth and fifth larval instars, the rate of which is modulated by environmental factors such as the light, diet, temperature and relative humidity (Shimizu, 1982). The morphological and anatomical changes in the silk gland are associated with concomitant changes in its biochemical constituents, including proteins. In view of its importance for silk production, the silk gland attracted the attention of several researchers, especially with regard to silk proteins. Many researchers have made efforts to answer questions about how physiological and biochemical mechanisms are responsible for these changes. A through understanding of these mechanisms requires comprehensive study. In this context, proteomics by the peptide mass fingerprinting method (PMF) using two dimensional electrophoresis, (MALDI- MS), and SDS PAGE is one of the more powerful tools used to identify various proteins rapidly and to monitor their changes.

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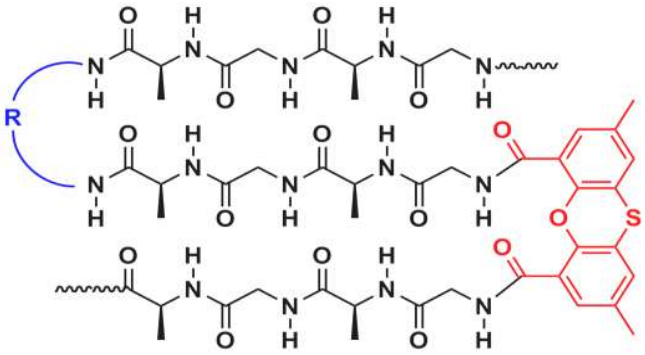
Silk constituents

Silk fibre is constituted by two important proteins, fibroin (73.5 %) and sericin (22.28 %). The chemical formulae of sericin is C15 H 25 N5 O 8 and that of fibroin is C 15 H 23 N 5 O6.A fibrous protein is composed of heavy (H) chain, Light (L) chain and glycoprotein linked by disulfide bonds. The second being sericin a natural macromolecular protein, serving as an adhesive to unite fibroin for making silk cocoons of silkworm, *B. mori*. Recently, silkworm is being used as biofactory for the production Protein.

(Anti parallel β-sheet structure of silk protein)



(Proposed secondary structure)

Isolation of silk proteins

Preparation of fibroin from larval silkglands

The fifth instar larvae of Bombyx mori were dissected and posterior silkglands were excised (Fig. 1). The posterior silkglands were opened in cold distilled water and the translucent viscous material was extruded out. The extruded insoluble materials were solubilized with 1% lithium dodecyl sulfate (LDS) in 10 mm Tris–HCl, pH 8. The solubilized silkgland protein was dialysed against several Changes of Buffer A. (5 M urea, 0.02 M Tris-HCl, pH 8.0) and was stored at -20ºC.

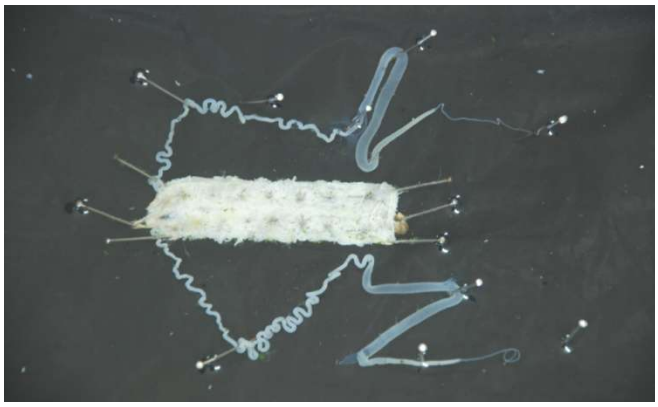


Fig. 1. Silkglands of silkworm *Bombyx mori*. Dissected from Fifth Instar larvae

Analysis of crude silkgland protein and its purification

Discontinuous 5% sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) was performed according to Laemmli (1970). About 0.2 ug of the major protein from gel filtration was incubated with sample loading buffer containing 10 mM 2-mercaptoethanol at 37ºC for 30 min. A similar amount (0.2 microgram) of 2-mercaptoethanol-treated and untreated samples were loaded on a 3% stacking gel cast at the top of a 5% SDS polyacrylamide gel and electrophoresed. After electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250 (Sigma) and destained in destaining solution (5% methanol, 7% acetic acid in water). The physiology of an insect is greatly altered during development from embryo to adult. For example, the complete metamorphosis exhibited by an insect such as the silkworm involves drastic changes. The growth of silk gland in silkworm is of paramount importance to the sericultural industry as it is responsible for the synthesis of silk proteins, the basic raw materials of the silk cocoon. Prominently, the silk gland grows during the fourth and fifth larval instars, the rate of which is modulated by environmental factors such as the light, diet, temperature and relative humidity (Shimizu, 1982). The morphological and anatomical changes in the silk gland are associated with concomitant changes in its biochemical constituents, including proteins. In view of its importance for silk production, the silk gland attracted the attention of several researchers, especially with regard to silk proteins. Many researchers have made efforts to answer questions about how physiological and biochemical mechanisms are responsible for these changes.

Preparation of fibroin from cocoons: The molecular heterogeneity of sericin was first demonstrated by analyzing native molecular proteins in the silk gland; Tashiro and Otsuki showed three kinds of sericin by ultracentrifugation, and Gamo separated atleast six sericins by SDS PAGE. When the silk protein solution obtained by dissolving fresh cocoons in aquous LisCN was analyzed by SDS PAGE for its proteome. Seven polypeptide bands, the molecular masses of which were from 20 to over 400KDa were observed. Among them the two dense bands above 300 and 20 KDa are derived from fibrion. Since the cocoon proteins after removal of sericin showed only the three bands corresponding to them (Fig 2.).

Fig. 2. Cocoons of Silkworm

Gel based proteomics of silk proteins

Himalayan states are abundant in insect fauna. Of the various insect species silk producing insects which include *Bombyx mori* and *Antherae mylitta* are very famous as these are highly beneficial to the mankind. They produce silk which is worldwide recognized as the Queen of fibres. The demand of silk is increasing in the world day by day and therefore many scientists are involved to increase the production and productivity of silk by using latest technologies. Natural fibres

have an edge over artificial fibres and silk excels all the fibres for a number of inherent characteristics such as lusture, softness, elegance, versatility, wearability, yarn strength etc. Silk fibre is constituted by two important proteins, fibroin (73.5 %) and sericin (22.28 %). These proteins are the complex organic nitrogenous substances. Proteins occupy a central position in the architecture as well as functioning of living matter. Proteomics is a newly emerging field of life science research, using high throughput technologies to display, identify or characterize all the proteins in a given cell, tissue or organism. Three approaches are mainly used in proteomics viz., Gel-based proteomics, mass spectrometry driven proteomics and protein arrays. Gel based proteomics is the older approach to screen the protein expression at large scale and it includes (IFF) First dimensional isoelectric focursing (Bravo *et al*., 1981) Second dimensional SDS-PAGE (Gorg *et al*., 1988) and micro sequencing.

Commercially important silks are produced by lepidopteran insects of the family Bombiciidae and Saturnidae. Silkworm is a good choice amongst laboratory species for research. More than 500 mutations have been recorded in the silkworm gene bank. Mass spectrometry driven technique for proteome analysis were carried out by Manan *et al*. (2001) and in *Bombyx mori* L*.,* proteins have been evaluated by Peptide Mass Fingerprinting Analysis by Zhang *et al*., 2005. Seven polypeptide bands, the molecular masses of which were from 20 to over 400 kDa were observed. Among them the two dense bands above 300 and 20 kDa are derived from fibrion. Isolation, purification and characterization of silk protein from cocoon peduncle of *Antherae mylitta* were carried out by Dash *et al*. (2006). A high molecular weight water soluble glue protein was identified in the cocoon peduncle with 200 kDa molecular weight.

The bands other than those derived from fibrion were considered to correspond to the main components of sericin. The largest polypeptide, which moved a little more slowly than fibrion heavy chains was estimated to have a molecular weight of around 400KDa.As it was considered to be the same component as seen in the MSG, so it was named as sericin M. The two close bands around 250KDa which were corresponding to be the product of alleles were named as sericin A, as their sizes agreed with the polypeptide which were specially found in the anterior part of the silkgland. The clear bands at about 150 KDa were found abundantly in the posterior part of the silkgland, hence named as sericin P. The two close bands around 250KDa which were corresponding to be the product of alleles were named as sericin A, as their sizes agreed with the polypeptide which were specially found in the anterior part of the silk gland (Takasu *et al.,* 2002). The clear bands at about 150 KDa were found abundantly in the posterior part of the silk gland, hence named as sericin P.

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