

Silk and its Biosynthesis in Silkworm *Bombyx mori*

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Abstract

Silk is the queen of textiles, a protein filament like other proteins collagen, elastin, keratin, sporgin etc. produced by several insects at their larval stages undergoing complete metamorphosis. It is a thin, long, light and soft fibre synthesized by silk gland cells of silkworm larva and stored in lumen of the gland; subsequently converted in to fibre. There are two different layers in the silk fibre i.e. sericin (outer layer) and fibroin (inner layer). Sericin is removed at the time of cocoon cooking and left fibroin, is the actual silk made up of two 'Brins' used as thread in different manufacturing process. This review mainly focuses on silk and its biosynthesis in silkworm *Bombyx mori*. Synthesis of silk is started after the 4th moult of the silkworm when the posterior silk gland cells are increased in size even though total number of cells remains constant throughout the postembryonic life. The process of biosynthesis has four steps i.e. Supply of amino acid, role of ribosomes, role of FmRNA and role of tRNA. The two threads coming from each gland, oozes out through spinneret in the form of a liquid, harden in contact with air and form the continuous thread. Its length varies from race to race i.e., 350 m in multivoltine race and 1600 to 1800 m in univoltine or bivoltine silkworm race of *Bombyx mori*.

Keywords: Silk, *Bombyx mori*, sericin, fibroin, silk gland, amino acid, protein synthesis.

Introduction

Silk is the most beautiful of all fibres, known as the queen of textiles, a protein filament like other proteins collagen, elastin, keratin, sporgin etc. which is an essential constituent of cocoons (Komatsu, 1975). Silks are produced by several insects at their larval stages undergoing complete metamorphosis, but some adult insects such as web spinners, bees, wasps, silverfish, mayflies, thrips, leafhoppers, beetles, lac wings and ants also known to produce little amount of silk throughout their lives. Commercial silks are produced by silkworm belongs to the families, viz., Saturniidae (*Samia ricini*, *Antheraea assama*, *Antheraea mylitta* and *Antheraea proylei*) and Bombycidae (*Bombyx mori*). *Bombyx mori* commonly known as mulberry silkworm, produce a delicate creamise white silk fibre which is the main commercial silk in the world.

Silk protein is synthesized by silk gland cells of silkworm larva and stored in lumen of the gland; subsequently it is converted into fibre. The fibre is thin, long, light and soft, well known for its water absorbency, dyeing affinity, thermo tolerances, insulation properties and lustre (Mondal *et al.*, 2007). It is the raw material for producing extensive fabrics, parachutes, different lining materials, artificial blood vessels and surgical sutures. Silkworms secrete silk as liquid during the process of spinning, it passes through the anterior gland and expelled out through the spinneret opening (Shimizu, 2000) and it becomes fibre after coming in to contact with air.

There are two different layers in the silk fibre i.e. sericin and fibroin. The outer sericin layer is insoluble in cold water; however, it is easily solubilized to smaller fractions in hot water (Gulrajani, 1988). Sericin is useful because of its special properties viz., resistance to oxidation, antibacterial properties and resistant to UV light. At the time of cocoon cooking, sericin is removed and leftover fibroin is the actual silk made up of two brins used as thread in different manufacturing process (Mondal *et al.*, 2007).

Composition of silk

Silk filament is a protein fibre composed of sericin and fibroin. Raw silk contains other natural impurities like fat and waxes, inorganic salts and coloring matter besides the proteins sericin and fibroin (Table 1).

Table 1. Composition of silk (*Bombyx mori*).

Composition	Percent (%)
Fibroin	70-80
Sericin	20-30
Wax matter	0.4-0.8
carbohydrates	1.2-1.6
Inorganic matter	0.7
Pigment	0.02
Total	100

Data based on Mondal *et al.* (2007).

Silk gland and protein synthesis

Silk gland is a paired exocrine gland, located at the two lateral sides under the alimentary canal of silkworm. Each gland is basically a tube like structure made up of glandular epithelium tissues. The cells of the silk gland constitute with extremely ramified nucleus containing numerous nucleoli. Nuclear ramification develops gradually as the larva grows and reaches prominent size in the late instars (IV and V). Silk gland is divided into three distinct regions. The posterior part is 15 cm long and is composed of about 500 secretory cells, which synthesize fibroin. The middle silk gland is about 7 cm long and contains about 300 secretory cells producing silk sericin; the protein, which cements the fibroin. The anterior part is a short tube only 2 cm in length composed of 250 cells. It is assumed that anterior region unites the sericin and fibroin layer prior to secretion. Silk gland of *Bombyx mori* secretes one fibroin and three layers of sericin from the each posterior, middle and anterior part respectively (Mondal *et al.*, 2007). The anterior ends of the anterior region of the gland join and open into mouth through a spinneret. A pair of labial glands present on the anterior silk gland function unites the two fine threads secreted by the two silk glands known as 'Brin' and forms 'Bave', the actual silk fibre (Akai *et al.*, 2005).

Structure and composition of silk protein

Fibroin: Fibroin is the principal building block of silk fibre secreted by the gland cells of the posterior silk gland. Fibroin's texture is highly affected by the crystalline structure that makes it durable. Fibroin constitutes about 70-80% of total composition of silk with lot of the amino acids. Fibroin contains large content of amino acid glycine, alanine followed by serine and tyrosine (Table 2). These 4 amino acids represent about 95% of the total proteins while rests are smaller amounts of vitamins, aspartic acid, glutamic acid etc. Basically fibroin is made up of two components viz. crystalline component (occupies two third) and amorphous component (occupies one third) of the fibroin molecule (Kamili and Masoodi, 2000). In crystalline component, amino acid are present in a definite manner with a definite space between them and the glycine residue repeatedly alternate with two other amino acids, more often with alanine followed by serine usually in the ratio of 3:2:1, throughout the sequence (Strydom *et al.*, 1977). The amino acid molecules of amorphous component are arranged irregularly with irregular spaces between them and usually with a tyrosine residue (Gage and Manning, 1988). The fibroin is synthesized in liquid form. It has two parts; the major homogeneous part known as Heavy chain (H-chain) with molecular weight 350 KDa and the minor heterogeneous part Light chain (L-chain) having molecular weight of 25 KDa. These two chains are connected by disulphide linkage (Shimura, 1988; Gopinathan, 1992). It is reported that H-gene is located on 25th chromosome and L-gene is located on the 24th chromosome of *B. mori* (Kamili and Masoodi, 2000).

Table 2. Amino acid composition of fibroin and sericin.

S.No.	Amino acid	Fibroin	Sericin
1	Glycine	44.4	14.7
2	Alanine	29.6	4.3
3	Serine	12.1	37.3
4	Tyrosine	5.2	2.5
5	Valine	2.2	3.5
6	Aspartic acid	1.3	14.8
7	Glutamic acid	1.0	3.4
8	Threonine	0.91	8.6
9	Leucine	0.53	1.4
10	Phenyl alanine	0.63	0.38
11	Proline	0.36	0.36
12	Methionine	0.63	0.76
13	Cystine	-	0.51
14	Lysine	0.32	2.4
15	Histidine	0.14	1.1
16	Arginine	0.47	3.5
17	Isoleucine	-	0.70
18	Tryptophan	-	0.20

Data based on Lucas *et al.* (1960); Kamatsu (1975); Shimura (1976; 1978; 1982) and Kamili and Masoodi (2000).

Sericin: Sericin is a hot water-soluble macromolecular globular protein with molecular mass of 10-310 kDa, cements the fibroin fibre that helps in the formation of cocoon. Sericin contributes about 20-30% of the total cocoon weight. Sericin is made up of 18 amino acids, most having strongly polar side groups such as hydroxyl, carboxyl and amino groups (Gulrajani, 1988). Sericin has three layers: i) Sericin A (Innermost layer), ii) Sericin B (middle layer) and iii) Sericin C (outermost layer). These layers are secreted by posterior, middle and anterior silk gland cell respectively and are piled one upon another around the central part (Prudhomme *et al.*, 1985).

Fibroin and sericin biosynthesis

After the 4th moult, the posterior silk gland cell increased in size even though the total number of cells remains constant throughout the postembryonic life. DNA reaches to maximum level and FmRNA (Fibroin messenger RNA) become active in the middle of 5th instars. Biosynthesis has four steps i.e., i) Supply of amino acid, ii) Role of ribosomes, iii) Role of FmRNA and iv) Role of tRNA (Kamili and Masoodi, 2000).

Supply of amino acid: Silk protein have very peculiar amino acid composition. During active feeding stage, most of the amino acids supply from digestion of feeding materials and transported to silk-gland. Towards the end of the larval life, silk synthesis is maintained by reserves amino from degenerating tissues of gut and integument (Naguchi *et al.*, 1974). The main amino acid, glycine, alanine, serine and tyrosine are synthesized in the silk-gland cell by transamination process (Prudhomme *et al.*, 1985).

Role of ribosome: Ribosome is the key element of fibroin synthesis and is fairly uniform in size and appearance. Ribosome composed of two subunits i.e., Larger and smaller subunit.

It is present both in free and attached with rough endoplasmic reticulum. Group of ribosomes are known as called polyribosome. Each polyribosome contains 45-112 numbers of ribosomes (Prudhomme *et al.*, 1985).

Role of FmRNA: The FmRNA plays an important role in silk synthesis. It carries coded message from DNA and arranges itself in an unoccupied ribosome. The code words are triplet known as codons carrying coded message for which has potential for reading and translating the coded message in complementary to the codons, known as anticodons (Kamili and Masoodi, 2000).

Role of tRNA: The tRNA is the smallest known RNA is an oligonucleotide, intermediate between activated amino acid and FmRNA (Kamili and Masoodi, 2000). It links the amino acids for the proteins thus, play an important role in the regulation the process of silk synthesis. The amino acids lie free in the cytoplasm and are taken to ribosome by tRNA. The selection of required amino acid is performed by tRNA with the enzyme amino acid tRNA synthetase (Prudhomme and Couble, 1979).

Pathway to making silk protein and secretion

There are four phases in protein synthesis i.e. are transcription, initiation, elongation and termination. Silk protein is released by polysome are taken in to cisternal space of rough endoplasmic reticulum migrate to golgi bodies then processed, packed and released as fibroin molecule. This molecule move to the apical surface through a well developed radical microtubular system lying deep in the cytoplasm and then taken to microvilli and secreted out into the lumen of the silk-gland (Sasaki *et al.*, 1981). When silkworms attain full growth, two silk-glands become filled by silk fluid, ultimately the ripe worms start spinning cocoon. Each posterior gland secretes silk fibroin pushed towards the middle gland where sericin cements the fibroin. The two threads coming from each gland are joined with the help of secretion which form the actual filament 'Bave', oozes out through spinneret in the form of a liquid. The silk fluid hardens in contact with air and form the continuous thread. Its length varies from race to race i.e., 350 m in multivoltine race and 1600-1800 m in univoltine or bivoltine race. Larva spins cocoon from exterior to interior in the form of '8' and turns about 6,000 to 3,00,000 times inside the cocoon for completing the process of spinning (Kamili and Masoodi, 2000).

Conclusion

For the production of a large amount of silk protein during a brief period in 5th instar, it is essential to supply sufficient amino acids. Among the amino acids, glycine is the key amino acid for controlling the synthesis of silk proteins. About 70% of glycine is synthesized in the silkworm tissues, presumably in the fat body and silk-gland.

Therefore, sufficient supply of feeding material i.e., fresh leaf can be possibly used to produce more amino acids that become the driving force for the synthesis of silk proteins ultimately the commercial raw silk.

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