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[Review]

The silk proteins, sericin and fibroin in silkworm, *Bombyx mori* Linn., - a review

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ABSTRACT

The domesticated silkworm, *Bombyx mori* Linn., a lepidopteran molecular model and an important economic insect that are emerging as an ideal molecular genetic resource for solving a broad range of biological problems. The silkworm, *B. mori* produces massive amount of silk proteins during the final stage of larval development. These proteins are stored in the middle silk gland and they are discharged through the anterior duct and spinneret, at the end of the fifth instar. Two kinds of silk proteins have been distinguished as major components of silk cocoons, the first being fibroin, a fibrous protein composed of heavy (H) chain, Light (L) chain and glycoprotein linked by disulfide bonds and 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 of useful protein using the silk gland, which has promoted the technological development in sericulture. With the above background silkworm can be classified as a value added biomaterial for medical application, application of silk protein fibroin and sericin as a biomaterial and other seri-byproducts. The present paper overviews some important studies carried out on sericin and fibroin of silkworm, *Bombyx mori* Linn.

Keywords: Silk protein, Sericin, Fibroin, SDS polyacrylamide gel electrophoresis, *Bombyx mori*.

INTRODUCTION

Insects mainly belong to two families, *viz.*, Saturnidae and Bombycidae, which spins silk fibre. *Bombyx mori* belongs to Bombycidae produces a delicate twin thread of silk fibroin, which is coated by a protective cover of sericin. Silk protein is a kind of protein like collagen, elastin, keratin, fibroin, sporgin *etc.*, is an essential constituent of cocoon filament (Komatsu, 1975).

The silk fiber protein is synthesized by silk gland cells and stored in the lumen of the silk glands. Subsequently, it is converted into silk fibres. When the silkworms secrete the liquid silk during the spinning, it passes through the anterior gland and expelled out through the spinneret opening (Shimizu, 2000). Quantity and nature of sericin are fundamental characteristics in conferring distinctive traits to the cocoon (Sadov *et al.*, 1987).

Sericin is insoluble in cold water, however, it is easily hydrolyzed, where by the long protein molecules brakes down to smaller fractions, which are easily dispersed, or solubilised in hot water (Gulrajani, 1988). Sericin protein is useful because of its special properties *viz.*, resists oxidation, antib acterial, UV resistant and absorbs and release moisture easily, inhibitory activity of tyrosine and kinase *etc.* (Fig 1). Sericin, a major component of silk fiber, has been selectively removed from fibroin during the silk manufacturing process to make silk lustrous and the removed sericin goes as waste material. Now a days Seri- waste products and Seri- byproducts are used as a value added products.

After Degumming, the leftover is fibroin made up of two brins. Silk fibre can be used for many purposes including textile, medical and industrial applications. The silk fibre is

thin, long, light and soft. It is well known for its water absorbency, dyeing affinity, thermo tolerances, insulation properties and luster (Fig 2). It is the raw material for producing precious fabrics, parachutes, tyre lining materials, artificial blood vessels and surgical sutures. Phillips *et al.*, (2005) reported that ionic liquid could hold the key to the production of designer silk fibers with enhanced mechanical and optical properties. The silk fibers have outstanding natural properties, which rival the most advanced synthetic polymers, yet unlike synthetic polymers the production of silk does not require harsh processing conditions. It is reported that the introduction of ionic liquids to silk processing opens an exciting avenue for controlling the microstructure to tune the macroscopic properties.

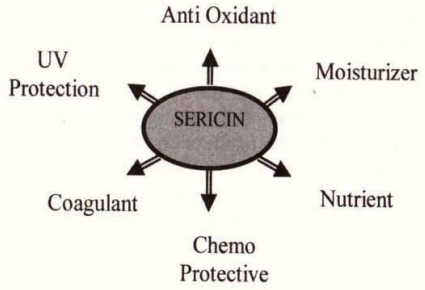

Fig 1. Diagrammatic representation of Attributes of sericin.

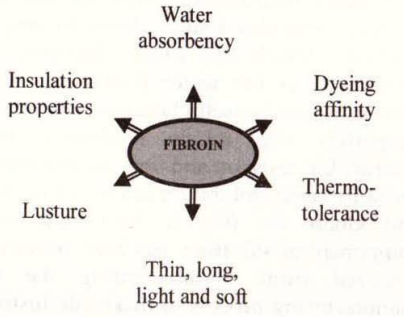

Fig 2. Diagrammatic representation of Attributes of Fibroin.

It is estimated that out of about 1 million tons (fresh weight) of cocoons produced world wide approximately 4, 00,000 tons of dry cocoon are generated, that have 50,000 tons of recoverable sericin. Indian production of 1,600 tons of silk can be source of about 250 to 300 tons of sericin per year (Gulrajani, 2005). If this sericin protein is recovered and recycled, it would be a significant economic and social benefit.

SILK GLAND

The natural silk synthesized by the silkworm and spun in the form of a silk cocoon is originally synthesized in the silk gland. Silk gland of *B. mori* is a typical exocrine gland secreting large amount of silk proteins. It is a paired organ consisting of modified labial/salivary glands located at the two lateral sides under the alimentary canal. Each gland is basically a tube made of glandular epithelium with two rows of cells surrounding the lumen.

The cells constituting the gland are huge polyploid cells each with extremely ramified nucleus containing numerous nucleoli. Nuclear ramification develops gradually as the larva grows and reaches conspicuous size in the 4th and 5th instars. Ramification considerably enlarges the nuclear surface and apparently facilitates the transfer of materials related to the silk synthesis between the nucleus and the cytoplasm. According to its morphology and function, the silk gland can be divided into three distinct regions (Fig 3). The posterior part, about 15 cm long and is composed of about 500 secretary cells, which synthesize silk fibroin.

The middle silk gland in the lumen of which silk proteins are stored until spinning, is about 7 cm long and contains about 300 secretory cells producing silk sericin, the protein which cements the fibroin thread of the cocoon. The anterior part about 2 cm long is a thin duct composed of about 250 cells with no known secretory function. Akai *et al.*, (2005) reported that the *Bombyx mori* silk gland secretes one fibroin and three layers of sericin from the each posterior and middle silk gland in a normal larva.

The Nd-sD mutant is silk fibroin secretion deficient. In the mutant, a disulfide linkage between the heavy (H) chain and light (L) chain is not formed because of partial deletion of the L-chain gene, which is essential for the intercellular transport and secretion of fibroin. To utilize the inactivity of the mutant L-chain, Inoue *et al.*, (2005) investigated the possibility of using the Nd-sD mutant for the efficient production of recombinants in the silkworm. The posterior

silk glands are not sufficiently developed and the liquid fibroin is scarcely secreted, but there is no such disorder in the middle silk glands. Yamamoto *et al.*, (2002) have introduced a new silkworm race which produces only sericin in Japan in the name of "Sericin Hope" introducing Nd-sD mutant. Feiying *et al.*, (2005) studied the analysis of protein variety of middle silk gland cells of the fifth instar larvae of silkworm, *B. mori* at different developmental stages.

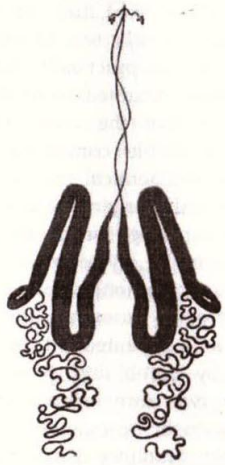

Fig 3. Schematic representation of silk gland of silkworm (*Bombyx mori* L).

The silk gland has the capacity to produce large amount of silk proteins. In order to use as a foreign proteins, *piggy*Bac vectors were developed to express transgenes in the silk gland using silk gene promoters to drive the expression of the integrated foreign genes. Several promoters were tested to synthesize foreign proteins like procollagen III or globular ones and successfully produced in the silk glands of *Bombyx mori* (Chavancy, 2005).

COMPOSITION OF THE COCOON FILAMENT

Gulrajani (1988) reported that the silk fiber is almost a pure protein fiber composed of two types of proteins *viz.*, sericin and fibroin. Sericin is chemically a non-filamentous protein. Besides sericin, raw silk also contain other natural impurities namely, fat and waxes, inorganic salts and colouring mater (Table 1). Rui (1998) studied the outer layer of the silk fiber and revealed that the sericin content is more in outer layer, where in fibroin content is less (Table 2).

Table 1. Composition of silk in *Bombyx mori* (Gulrajani, 1988).

Component	%
Fibroin	70-80
Sericin	20-30
Wax matter	0.4-0.8
Carbohydrates	1.2-1.6
Inorganic matter	0.7
Pigment	0.2
Total	100

Table 2. Change in cocoon filament of a particular silkworm having dissimilar layers (Rui, 1998).

Layer	Fibroin (%)	Sericin (%)	Ether soaked substance (%)	Ash content (%)
1	64.94	32.41	1.36	1.23
2	74.92	23.15	0.84	1.09
3	78.34	19.79	0.77	1.07
4	79.69	17.86	1.32	1.15
5	79.09	17.78	1.75	1.39

SILK PROTEIN FROM THE SILK GLAND

Silk fibroin secreted in the lumen of posterior silk gland (PSG) of *B. mori* consists of three protein component: High (H)-chain 350 k Da (Shimura *et al.*, 1982, Zhou *et al.*, 2000), Low (L) - chain 26 k Da (Yamaguchi *et al.*, 1989), and Glycoprotein P25 30 k Da (Chevillard *et al.*, 1986a, 1986b). These three types of fibroin (H-chain, L-chain and P 25) are common among different silk producing insects in Lepidoptera, although the fibroin of Saturnidae species secreted as dimer of H-chain.

Quantitative enzyme linked immune-osorbent assay (ELISA) with specific antibody for each protein component showed that the molar ratio of H-chain, L-chain, and P 25 is 6:6:1 for the fibroin secreted into the posterior silk gland (PSG). The N-linked oligosaccharide chain of P25 has been suggested to be involved in the later interactions (Inoue *et al.*, 2000). Fibrohexamer was proposed as a functional name of P25

and its central role is maintaining the structure of elementary unit. The disulfide linkage between Cys-172 of the L-chain and Cys-c20 of the H-chain (Tanaka *et al.*, 1999) is not responsible for the maintenance of the once formed elementary unit (Inoue *et al.*, 2000). But the H-L linkage is essential for the large-scale production of fibroin (Mori *et al.*, 1995), because fibroin is retained in endoplasmic reticulum in the absence of disulfide linkage between the H and L-chains (Gamo and Sato, 1985). Akai *et al.*, (1987) observed the fine structural change of liquid columnar fibroin in the lumen of silk gland during the passage from the posterior to the anterior silk gland during the spinning stage, in order to analyze the mechanism involved in the formation of a single cocoon filament. In the posterior part of the posterior silk gland, the columnar fibroin located in the lumen consisted numerous spherical masses of fibroin fibers (MFFs).

These MFFs adhered closely together showing a higher concentration in the posterior part of the middle silk gland, and become homogeneous and compact in the anterior silk gland. By the observation of various portion of silk gland, it is concluded that the cocoon filament is composed of oriented elementary fibroin fibers and these fibers are derived from MFFs as they undergo structural change during the passage through the silk gland lumen. Komatsu (1975) postulated molecular aggregating structure of sericin and its changes.

The part of the liquid sericin in the middle silk gland that is easily crystallized by drying is possibly made of amino acid residues with short side-chains and is folded into the globular matrix made of stretches with longer side-chains, crystallizing less readily and on drying, become film of unoriented crystal structure. When this film is swollen in water, stretched and dried, it changes to oriented fiber structure. However, since the orientation is unstable by hot water treatment and thus there is a reversible relationship between the orientation and non-orientation.

X - RAY DIFFRACTION ANALYSIS

Konishi (2000) studied the X ray diffraction of fibroin and noticed 1 mm thick parallel silk fibroin fibres. It is observed that fibroin consists of non- crystalline and crystalline regions. The crystalline region tends to be oriented along the fibre axis because the fibre is drawn as it is extruded from the spinnerets of the silkworm.

Tsukada (1983) studied the crystalline structure and molecular conformation of silk sericin, by means of X ray diffraction, infrared spectrometry and differential scanning calorimetry of the wild silk (*Antheraea pernyi*, *Antheraea yamamai*, *Philosomia cynthia ricini*) by boiling in water. The crystalline structure and molecular conformation of silk sericin obtained from wild silks is not practically different from that of sericin obtained from *B. mori*. Only difference is that the wild silk sericin is relatively insoluble compared to *B. mori*, mainly due to chemical interaction between silk sericin and inorganic minor components or tannins contained originally in wild silk. The structure of a crystalline form of *B. mori* silk fibroin commonly found before the spinning process (known as silk I) has been proposed as a repeated β-turn type II- like structure by combining β obtained from solid-state two dimensional spin-diffusion nuclear magnetic resonance and rotational-echo double resonance (Asakura *et al.*, 2001). The molecular and crystal structure of the crystalline modifications of *B. mori*, silk I, is determined by X- ray diffraction method. According to Kenji *et al.*, (2001), the cell dimensions are essentially the same as those found in the synthetic model polypeptide (L-Ala-Gly). The (phi, pI) values of L-Ala and Glee in the repeating unit (-112°,-6°C), (71°, -99°C) which are in the bridge and fourth quadrant regions of the Ramchandran map, respectively.

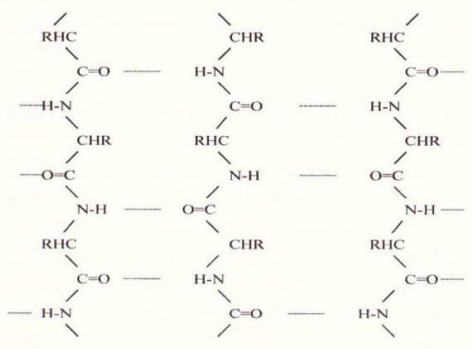

Fig 4. Crystalline structure of the peptide chains in silk fibroin.

The observed molecular conformation has a "crank-shaft" or a S- shaped zigzag arrangement, leading to remarkable agreement of observed and calculated structure amplitudes of both dipeptide and hexapeptide sequences, and has a reasonable hydrogen bond networks. X ray structure analyses of the crystalline domains of fibroin show that the peptide chains pack in fully extended forms (Fig 4).

MOLECULAR CONFORMATION OF SERICIN AND ITS TRANSITION

Iizuka (1969) reported that sericin extracted from the liquid silk and fresh cocoon shells of a silkworm mutant, which secretes only sericin, is a random coil with 5-10 % β-structure and no α-helix. Komatsu (1975) also reported that sericin, in aqueous solution, displays both random coil and β-structure, but lacks α-helix. He studied the sericins from both the liquid silk in the silk gland and the cocoon filament and confirmed the relationship between solubility in hot water and molecular conformation (Komatsu , 1982). To date, most experimental evidences indicate that sericin exists mainly in the random coil or β-structure. It is believed that β structure is intrinsic to liquid silk.

Though, these reports are not in conformity with Tsukada (1983) based on analysis of circular dichroism spectrum, which, indicated that sericin extracted from liquid silk for 45 minutes with water contains a small fraction (10%) of α-helix.

Komatsu (1980) argued that during the dissolution of the liquid silk in water, part of the sericin IV become a white suspension due to the coexisting cocoon yarn wax but does not coagulate and the β structure is originally present in the liquid silk. β-structure sericin is more insoluble than random coil sericin. The transition of sericin from its random coil to β- structure takes place by repeated absorption and de-absorption of moisture and by heating during the absorbed state *i.e.*, hygroscopic conditions (Komatsu, 1980). Ishikawa and Hirabayashi (1968) reported that sericins have a cross β structure when a water solution of sericin containing 50% dioxane (Iizuka, 1969) or methanol (Ishikawa *et al.*, 1987).

Komatsu (1975) believed that the sericin fraction closest to fibroin in the cocoon filament has molecular chains also arranged in cross β structure, rather than having the main axis of crystallites oriented at right angle to the fiber axis. The intra- molecular bonds of sericin having random coils are broken by the absorption of water molecules and the folded structure becomes unfolded into an extended structure and is transformed into a β structure.

β Structure is more stable regarding energy. Part of the sericin thus transformed into β structure is fixed by its new intermolecular hydrogen bonds and remains crystallized even when the water molecules are removed by drying.In a new cycle of water absorption, the crystalline structure already formed, remains unaffected, whereas , a further fraction of random coils state crystalizes into a β structure there by increasing the portion of β structure and promotes crystalization. Heating during moisture absorption activates the thermal motions of segments and accelerates transformation. Thus the sericin gets modified in the direction of difficult solubility due to repeated moisture absorption and loss.

SERICIN LAYER STRUCTURE OR SERICIN FRACTION

Scientists have separated sericin of cocoon shell into two proportions: (1) α-sericin and (2) β-sericin. α- Sericin is present in the outer layer of cocoon shell and β-sericin in the inner layer. The α-sericin contains lesser C and H and somewhat more N and O than the β-sericin (Bose *et al.*, 1989). The solubility of α-sericin in the boiling water is more than β-sericin. Sadov *et al.*, (1987) have come to the conclusion that native sericin is mixture of two substances, sericin A and Sericin B.

Sericin in aqueous solutions obtained on degumming silk is not an individual chemical substance but is a mixture of at least two substance, fraction A can be separated by fractional precipitation by adding 15g of ammonium sulphate per 100 ml of sericin solution. When ammonium sulphate is added to the filtrate, sericin B will be precipitated. Some scientists described that on the cocoon thread, the sericin layers are formed from the outside to inside in the order of I, II, III to cover the fibroin. Three fractions of sericin which were different in solubility and called them sericin fractions I,

II, and III based on the order of ease of dissolution in hot water and that their ratios were approximately 40:40:20. Based on the different degree of solubility, sericin layers can be called as Sericin A and Sericin B or Sericin □ and □ or Sericin I, II, and III. The sericin A is more soluble than the others. It is found that of the sericin I, on the cocoon filament is amorphous where as II and III are crystalline. Komatsu (1975) observed that four fractions of sericin viz., I, II, III and IV are having different solubilities harder to dissolve being sericin IV. Fourth fraction of sericin was reported to have higher specific gravity and crystallinity than moisture content (Table 3).

Robson (1985) reported that sericin may be separated into sericin I, II, III and IV by their different solubilities in hot water and assessing the degree of solubility by UV absorption. The greatest sericin content is present in the outer layer of cocoon whereas the least sericin proportion is present in the innermost layer of a cocoon. Fibroin is the principal water insoluble protein (i.e., 78% of the weight of raw silk).

AMINO ACID COMPOSITION IN SERICIN AND FIBROIN

Yamada (1978) indicated that the sericin of mulberry wild silkworm, *Bombyx mori mandarina*, seem to contain the same kind of amino acids as the domesticated silkworm, *B. mori*. However, the wild silkworm sericin contained serine, proline, methoinine, glucosamine, galactosamine and histidine in lower amount and throeonine, glutamic acid, cystine and phenyl amine in higher amount. The content of threonine, galactosamine and glucosamine were significantly higher in the inner layer of cocoons than in the outer layer. Further more, the sericin extracted from the floss showed high contents of serine, glycine, valine and tyrosine but low contents in threonine, aspartic acid, alanine, cystine, leucine, glucosamine, galactosamine, lysine and histidine as compared with the sericin of cocoon layer (Table 4).

Table 3. Properties of sericin fractionated with hot water (Komatsu, 1975).

	Sericin				Total sericin
	I	II	II	IV	
Content (%)	41.00	38.60	17.60	3.10	100.00
Co-efficient of dissolution velocity	5.33	1.76	0.70	0.22	-
Moisture regain (%)	16.70	16.20	15.70	14.50	16.30
Specific gravity	1.400	1.403	1.408	1.412	1.407
Crystallinity (%)	3.00	18.20	32.50	37.60	15.06

Table 4. Amino acid composition of sericin obtained from cocoon layer and floss of the mulberry wild silkworm (Yamada, 1978).

Amino acid	Cocoon layer			Floss
	Inner	Outer layer	Whole	
Aspertic acid	18.61	18.30	18.46	10.20
Threonine	11.39	8.44	9.92	6.29
Serine	28.12	29.05	28.58	40.28
Glutamic acid	4.90	4.78	4.84	4.31
Proline	0.51	0.56	0.53	0.66
Glycine	16.90	16.70	16.80	18.17
Alanine	4.84	5.15	5.00	4.43
Cystine	0.42	0.64	0.53	trace
Valine	2.67	2.91	2.79	3.46
Methionine	0.10	0.11	0.10	0.12
Isoleucine	0.60	0.67	0.63	0.67
Leucine	0.90	1.17	1.03	0.85
Tryrosine	3.28	3.39	3.33	4.09
Phenylamine	0.42	0.47	0.44	0.43
Lysine	2.26	2.89	2.58	1.89
Histidine	0.89	0.99	0.94	0.68
Arginine	3.03	3.41	3.22	3.30

The wild silkworm sericin contained more amino acids with nonpolar side chain and less amino acid with polar side chain than the domesticated silkworm sericin (Table 5). The co-efficient of pattern-similarity in amino acid composition was high between the sericin of wild silkworm and domesticated silkworms, while sericin of the wild silkworm such as *Antheraea* or *Phylosamia* showed significantly low similarity (Table 6). The results show that the amino acid composition of sericin extracted from the cocoon is species specific. Fibroin has high proportions of alanine, glycine and serine. A small amount of cystine residues give a very small amount of sulphar in the fibre. Fibroin contains only a small amount of amino acids, which have acid side chains (Table 7). The isoelectric point of silk fibre is around pH 5. There is low proportion of amino acid residues with large chains in silk.

SDS POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS - PAGE) OF SERICIN AND FIBROIN PROTEIN

Kurioka and Yamazaki, (2002) purified protein with a low molecular mass of 6027 from the cocoon shell of silkworm, *Bombyx mori*. Two dimensional polyacrylamide gel electrophoresis (2D-PAGE) resolved this protein into a single spot with pI 4.3 and Mr 6000. Amino acid sequence analysis revealed that this protein consists of 55 amino acids, six of these being cystine residues and is highly homologous to bovine pancreatic trypsin inhibitor type. Feiying *et al.*, (2005) uses the two dimensional Polyacrylamide gel electrophoresis (2D-PAGE) and the image analyses technology. The result indicated a significant difference in the protein composition of the same part of middle silk gland (MSG) cells among the second day, the fourth day and the mature silkworm of the fifth instar larvae of silkworm. Eight special protein spots are expressed in anterior part of MSG cells in the second day, sixteen in the fourth day of fifth instar and twenty-four in the mature silkworm.

Nine special protein spots are detected in the middle part of MSG cells in the second day, thirty-three in the fourth day of fifth instar and thirty-four in the mature silkworm. Ten special protein spots are found in posterior part of MSG cell in the second day, seven in the fourth day of fifth instar and twenty-five in the mature silkworm. Jain *et al.* (2005) analyzed 2D-PAGE patterns of protein from posterior silk gland of 4th day silkworm in 5th instar of different breeds of *B. mori*.

Protein forms of posterior silk gland are apparently different for different breeds and different protein spots are found when they compared to middle silk gland of different breed, implying that the transcription, translation of fibroin gene may involve a complex regulation system with great deal of regulation points. The qualitative and quantitative differences in proteins expressed in the middle silk glands of male and female silkworm larvae that differential silk colour are investigated by high resolution two-dimensional polyacrylamide gel electrop horesis (2-D PAGE). They reported that there are no distinct differential proteins between the silk gland tissue of female and male larvae within the same variety of sex- limited character.

Table 5. Content of amino acids with polar and nonpolar side chains constituting in the cocoon sericins from wild and the domestic silkworm (Yamada, 1980).

Groupof amino acid	Mulberry wild silkworm			Domestic silkworm	
	Inner cocoon layer	Outer cocoon layer	Floss	Inner cocoon layer	Outer cocoon layer
Polar	72.49	71.25	70.04	76.92	77.31
Hydroxyl	42.80	40.88	50.66	41.36	43.69
Acidic	23.51	23.08	14.51	28.45	25.16
Basic	6.18	7.26	5.87	7.11	8.46
Nonpolar	27.36	28.32	28.79	22.75	21.78
Ratio (Polar / Nonpolar)	2.65	2.51	2.43	3.38	3.35

Values of the domestic silkworms are given as mean of 8 varieties.
Contents are express as mol %.

Table 6. Pattern- similarity coefficient of amino acid composition of the sericin from mulberry wild silkworm (A) and other silkworms (B) (Yamada, 1978).

Silkworm	Pattern-similarity coefficient
Bombyx mori mandarina	1.000
Bombyx mori	0.990
Antherea yamamai	0.970
Antheraea pernyi	0.977
A. paphia mylitta	0.946
A. assamensis	0.974
Philosomia cynthia ricini	0.976

Pattern-similarity coefficient=S (A, B) =

$$\cos\theta = \frac{\sum_{i=1}^{n} A_i.B_i}{\sqrt{\sum_{i=1}^{n} A_{i_2}}\sqrt{\sum_{i=1}^{n} B_{i_2}}}$$

Table 7. Amino acid composition of silk fibroins (residues/ 1000 residues) (Robson 1985).

Amino acids	*B. mori* fibre
Glycine	446.0
Alanine	294.0
Valine	22.0
Leucine	5.3
Isoleucine	6.6
Serine	121.0
Threonine	9.1
Aspartic acid	13.0
Glutamic acid	10.2
Lysine	3.2
Arginine	4.7
Histidine	1.4
Tyrosine	51.7
Phenylalanine	6.3
Proline	3.6
Tryptophan	1.1
Methionine	1.0
(Cysteine)2	2.0
	Gly>Ala

Isolation of the smallest component of silk

Tokutake (1980) reported a series of polymers of the smallest component, detected by polyacrylamide gel electrophoresis, could be converted into the smallest component by reduction and aminoethylation. Fibroin and sericin fraction were separated by precipitation of sericin at pH 5.5. On the gel electrophoresis, sericin showed distinct band but fibroin did not. The component of the fibroin and sericin are fractionated by gel filtration on sepharose 6B. The smallest component in the sericin fraction is purified by re-chromatography and showed a single band on gel electrophoresis. Silk protein solution prepared by solubilization of a whole cocoon with ED/Cu (0.13 M Ethylenediamine/ 0.06 M-cupric hydroxide) solution showed distinct bands on polyacrylamide gel electrophoresis in the presence and absence of SDS, and that there exists a series of polymers.

These polymers are reduced by 2-mercaptoethanol to the smallest component of silk protein, which had an apparent mol wt of 24000 (Tokutake, 1980). Thus fibroin and sericin can be separated by precipitation then both the fraction of silk can be examined by polyacrylamide gel electrophoresis. The amino acid compositions of two fractions are shown in Table 8.

The supernatant fraction known as fibroin is particularly rich in glycine and alanine, which together accounted for 78-mol%. On the other hand, the precipitate fraction is known as sericin, which was rich in glycine, serine, alanine and aspertic acid, the combined contents of which amounted to 72-mol%. The results of electrophoresis suggest that components of sericin have define molecular weights but those of fibroin do not.

APPLICATION OF SERICIN AND FIBROIN PROTEIN

Silkworm is being used as biofactory for the production of useful protein. Silk proteins are natural polymers and are biodegradable with reactive functional groups that open up possibility to be cross-linked with other polymers to be used in controlled delivery. Like other common biomedical textiles such as polyester, silk contains various polar functional groups that might enhance antibiotic absorption.

Biodegradable materials

Environment - friendly biodegradable polymers can be produced by blending sericin with other resins (Annamaria *et al.*, 1998).

Table 8. Amino acid composition of supernatant fraction, the precipitate fraction and the smallest component of silk protein (Tokutake, 1980).

Proteins	Supernatant Fraction Fibroin	Precipitate Fraction Sericin	Smallest component
Aspartic acid	1.68	12.9	15.2
Threonine	1.17	5.21	3.66
Serine	9.48	18.9	12.0
Glutamic acid	0.96	4.25	7.50
Proline	0.00	0.69	2.49
Glycine	46.4	24.2	14.7
Alanine	31.6	15.2	13.2
Valine	2.04	3.34	5.54
Methionine	0.00	0.11	0.00
Isoleucine	0.28	1.82	5.50
Leucine	0.22	1.99	5.45
Tryrosine	4.98	4.10	3.70
Phenylamine	0.00	0.69	2.46
Lysine	0.58	2.07	2.03
Histidine	0.08	0.98	1.45
Arginine	0.29	3.02	3.76
AminoEthylcysteine	0.00	Trace	0.55

Contents are express as mol %.

The Polyurethane foams incorporating sericin are said to have excellent moisture-absorbing and desorbing properties (Minoura *et al.,* 1995). Polymer films, foams, molding resins, and fibers containing sericin (0.01-50% w/w) can be produce by reacting a composition comprising a polyol, tolylene, di- isocyanet, di-butyltin di-laurate (catalyst) and trichloromonofluoromethane (a blowing agent) in the presence of sericin. The moister absorption/desorption rate of the sericin containing polyurethane form is two-to five fold grater than that of control. Other procedures have also been reported for producing sericin-containing polyurethane with excellent mechanical and thermal properties (Hatakeyama, 1996).

Membrane materials

Membrane based separations (*e.g.*, reverse osmosis, dialysis, ultra filtration and micro filtration) are used in process such as desalination of water, production of extremely pure water, the bioprocessing industry and some chemical processes. Pure sericin not easily made into membranes, but membranes of sericin cross-linked, blended, or copolymerized with other substance are made readily, because sericin contains large amount of amino acid with neutral polar functional groups. Sericin and fibroin can be used to make membranes for use in separation processes. The insolubilized silk fibroin membrane could be used to separate the mixture of water and alcohol (Chisti, 1998). Mizoguchi *et al.,* (1991) describe a cross- linked thin film made of sericin for use as a separating membrane for water and ethanol. Sericin containing membranes are quite hydrophilic. Acrylonitrile used in making certain synthetic polymers can be copolymerized with sericin to prepare a protein containing synthetic polymer film for separating water from organics (Yamada and Fuwa 1994, Yamada *et al.,* 1993).

Functional biomaterials

Nakajima (1994) has found that sericin film located on lay of liquid crystal can uniformly orient the liquid crystal molecules to provide distortion-free high- quality crystal displays. Sericin- coated film is used on the surface of refrigeration equipment because of its antifrosting action (Tanaka and Mizuno, 2001). Use of the coated sericin film is an effective antifrosting method that can be

widely applied to refrigerators, deep freezers and refrigerated trucks and ships. Moreover use of the coated film on roads and roof can prevent frost damage. Sericin protein can be coated on surfaces of various durable materials to enhance functionality (Li, 1996). Sericin can be used in preparation of art pigments and for surface protection of articles. The material coated the sericin have excellent weather ability, good permeability and do not warp on drying. Sericin blends with water- soluble polymers, especially with polyvinyl alcohol (PVA). A blended hydrogel made of sericin and fibroin and PVA is said to have excellent moisture absorbing and desorbing properties and elasticity (Yoshii *et al.*, 2000). The hydrogel can be used as a soil conditioner and in medical materials and wound dressing. Miyairi and Sugiura (1978) reported a cross-linked sericin film for enzyme immobilization with glutaraldehyde as the cross- linking agent. The heat stability, the electro-osmosis resistance and the stability of the immobilized β-glucosidase on the cross-linked sericin film are higher than the free enzyme.

Medical biomaterials

Silkworm silk fibers have been the primary silk- like material used in biomedical applications particularly as sutures. During decades of use, silk fibres have proven to be effective in many clinical applications. At the same time, some biological responses to the protein have raised questions about biocompatibility. Tasubouchi (1999a) developed a silk fibroin- based wound dressing that could accelerate healing and could be peeled off without damaging the newly formed skin. The non-crystalline fibroin film of the wound dressing had a water content of 3-16% and a thickness of 10- 100 µm. Subsequently, the wound dressing was made with a mixture of both fibroin and sericin (Tsubouchi, 1999b). A membrane composed of sericin and fibroin is an effective substrate for the proliferation of adherent animal cells and can be used as a substitute for collagen. Minoura *et al.*, (1995) and Tsukada *et al.*, (1999) investigated the attachment and growth of animal cells on films made of sericin and fibroin. Cell attachment and growth were dependent on maintaining a minimum of around 90% sericin in the composite membrane. Film made of sericin and fibroin has excellent oxygen permeability and is similar to human cornea in its functional properties. It hoped that the sericin- fibroin blended film could be used to form article corneas (Murase, 1994). A novel mucoadhesive polymer has been prepared by template polymerization of acrylic acid in the presence of silk sericin (*Ahn et al.*, 2001). Silk protein can be made into a biomaterial with anticoagulant properties, by a sulfonation treatment of sericin and fibroin (Tamada, 1997).

Kato *et al.* (1998) provided the first evidence of antioxidant action of the silk protein by showing that sericin suppressed in vitro lipid peroxidation. Furthermore, sericin also found to inhibit tyrosinase activity. These results suggested that sericin is the valuable natural ingredient for food and cosmetics. The biopolymer sericin has a strong affinity to keratin.

Excessive transepidermal water loss (TEWL) is one of the causes of dry skin and skin moisturizers have been used to overcome it. The silk sericin has resemblance with the natural moisturizing factor (NMR). Sericin gel is prepared by using sericin solution with pluronic and carbopol as a stabilizer to prevent water loss from the upper layer of the skin. It forms a moisturizing, semi-occlusive, protective, anti-wrinkle film on the skin surface imparting an immediate, long lasting, smooth, silky feeling (Padamwar *et al.*, 2005). The configuration of sericin is very close to the one of human beings. That is why sericin can naturally saturate into skin and revitalize cells. It is discovered that sericin can restrain the functions of active-oxygen (major factor of aging), which brings wrinkles and dark spots.

The use of oxygen-permeable membranes from silk fibroin and silk sericin, containing about 60% water for contact lens, artificial skin, etc. The other uses of sericin includes, as a soil conditioner, coagulant for purification of waste waters, hygroscopic moisture-releasing polyurethane foams and their manufacture for furniture and interior materials, as additives for health foods to prevent colon cancers, medical composites of sericin, additives to rice cooking, fabric care compositions, light and sunscreen comp ositions, foam-forming aerosol shaving gels, sericin-coated powders for cosmetics, as dermatitis inhibitor, as wound protection

film, nail cosmetics, and chewing gums (Gulrajani, 2005).Fibroin has been explored as a biomedicine for various applications. Fibroin powder was processed in such a way to retain its natural, optical beauty. A unique property of this silk powder is its ability to hold and release moisture depending on the temperature and humidity of the surroundings. The extremely fine powder (11.3 μ size) is particularly ideal for applications in pressed powders, blusher, eye make up, lipstick and nail enamel.

Sericin and fibroin have been recently explored in the field of drug delivery systems. Wu *et al.*, (1996) studied the properties and application of wound protective membrane made by silk fibroin. It is concluded that the fibroin membrane has good wound healing properties. The fibroin hydrogels prepared either by treating a 2% (w/v) silk fibroin aqueous solution at 4 °C temperature or by adding 30% glycerol could be used as scaffolds able to promote in situ bone regeneration (Matta *et al.*, 2004). Using fibroin controlled release tablets, gels and mesosphere have been prepared. The applicability of fibroin, a major silk protein, to controlled release type dosage tablets is investigated in vitro and in vivo. The sulfated silk fibroins have anti-HIV-1 activity in vitro, apparently due to interference with the adsorption of virus particles to CD4+ cells, and completely blocked virus binding to the cells at a concentration of 100 micro gm/ml (Gotoh *et al.*, 2000).

The silk fibroin can be used as the substratum for the culture of animal cells in place of collagen (Inouye et *al.*, 1998). Aslani and Eral, (1994) investigated the uranium recovery from dilute aqueous solutions using silk fibroin. The aqueous solution of fibroin is used to prepare a membrane for immobilization of *Aspergillus niger* glucose-oxidase and *Pseudomonas fluorescens* lyophilized cells (Demura *et al.*, 1989). Yoshimura *et al.*, (1989) reported that the fibroin membrane is used to immobilizing coenzed insect cell culture as a vaccine.

Hu, (2006) reported that the Recombinant human-like collagen (RHLC) is blended with fibroin to prepare a novel biocompatible film as a scaffold material for hepatic tissue engineering applications. Solution blending is used to incorporate RHLC with silk fibroin to enhance the blend films biocompatibility and hydrophilicity while maintaining elasticity. Soluble fibroin enhances Insulin sensitivity and glucose metabolism in 3T3-L1 Adiposities. The fibroin protein is one kind of biological materials used for artificial skin and others medical application. Silk fibroin membrane supports the application as photo sensor for hydrogen peroxide analysis. Silk protein sericin, suppress DMBA-TPA induced mouse skin tumor genesis by reducing oxidative stress, inflammatory responses and endogenous tumor promoter TNF-alpha (Zhaorigetu *et al.*, 2003).

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