Chemical Structure and Physical Properties of *Antheraea assama* Silk

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SYNOPSIS

This work deals with the chemical composition, physical structure, and thermal behavior of *Antheraea assama* silk fibers. The amino acid composition is characterized by the high content of alanine, glycine, and serine. Among the amino acid residues with polar side chains, aspartic acid, arginine, and tyrosine prevail. The birefringence and isotropic refractive index values are 0.030 and 1.557, respectively. The stress–strain curve shows a yield point at 5% elongation, followed by a region of gradual extensibility. Elongation at break is about 40%. The X-ray diffraction pattern is typical of β silks, with $-(\text{ala})_n-$ repeats in the polypeptide sequences of the crystalline regions. The differential scanning calorimetry curve shows two minor endotherms at 230 and 300°C, before final thermal decomposition at 362°C. The maximum extent of contraction exhibited by the thermomechanical analysis curve is 2%. The surface of degummed fibers shows the presence of longitudinal striations. The fiber cross section is mostly elliptical. Raw fibers taken from cocoons show the presence of several crystalline deposits on the surface, identified as crystals of calcium oxalate.

INTRODUCTION

The large group of silk-producing organisms includes many species of Lepidoptera and Arthropoda. Early studies have been devoted to the analysis of the chemical and physical structure of most silks produced by insects and spiders, in an attempt to establish correlations between chemical composition, crystal structure, and biological classification.[1-4]

Antheraea silkworms belong to the family Saturniidae and live in various eastern countries, such as India and China. Wild cocoons are a source of textile fibers. Research has been recently undertaken in order to introduce modern rearing techniques, for example, making use of the newly developed artificial diet to improve reeling technologies.[5]

Among wild silks, that produced by *Antheraea pernyi,* also known as tussah silk is the leader in production and use. The silk produced by *Antheraea assama,* known as Muga, originates from India and is second in importance to tussah silk. Cocoons are large (about 5 × 2.5 cm in size) and exhibit a bright brown color. The eggs hatch 3–5 times a year and their size is smaller than that of other wild silkworms. Cellular systems have been developed for indoor rearing of *A. assama* silkworms,[6] the first successful attempt of mass rearing in controlled conditions. No detailed and exhaustive investigations on the physical and chemical structure of Muga silk have been reported. Bath et al.[7] reported crystallinity data, determined by X-ray diffraction and infrared spectroscopy, of Muga silk fibroin and residues obtained after partial hydrolysis.

Physical properties and chemical reactivity of silk fibroin are subjects of both fundamental and applied research for textile materials and biotechnological and biomedical applications. Silk has been used as a substrate for enzyme immobilization[8,9] and for the production of oxygen-permeable membranes.[10,11]

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Blood compatibility of *Bombyx mori* silk fibroin has been recently reported.[12]

One of the main interests of our research is the characterization of naturally occurring fibrous polymers. Physical properties, thermal behavior, and reactivity of both *B. mori* and *A. pernyi* silk fibers toward various modifying agents have been reported.[13–16] Moreover, we recently studied the chemical composition and physical properties of *Gonometa rufobrunnae* silk, a fiber produced by a silkworm belonging to the family Lasiocampidae.[17]

The aim of this study is to contribute basic knowledge on the chemical composition, physical structure, and thermal behavior of *A. assama* silk fibroin. The results may allow a better technological exploitation of this interesting textile fiber, as well as extend our knowledge of the relationship between structure and functional properties of natural fibrous polymers.

EXPERIMENTAL

Materials

The cocoons from *A. assama* silkworm were obtained from the Regional Muga Research Station, Central Silk Board, Government of India, Mirza. The *A. assama* silkworms were reared on Soalu plant leaves (*Litsaea polyantha*). The cocoon fibers were degummed according to methods in the literature,[7] by means of hot alkaline solution.

Measurements

Degummed silk fibers from *A. assama* cocoons were hydrolyzed by heating at 110°C for 20 h under vacuum in HCl 6*N*. The hydrolyzed samples were filtered through a glass disk, dried in a rotary evaporator at 40°C, and dissolved in Na citrate buffer 0.05*M* pH 2.2 (1 mg/mL). The amino acid composition was determined with a Hitachi L-8500 Rapid Amino Acid Analyzer.

The refractive indices parallel and perpendicular to the fiber axis were measured with the Becke's line method using a polarized microscope under the monochromatic light (Na light) at 20°C and 65% relative humidity (RH) according to published procedures.[18]

The tensile properties were measured with a Tensilon UTM-II tensile tester (Toyo Boldwin Co.) in standard conditions at 20°C and 65% RH.

Wide-angle X-ray diffraction (WAXD) profiles were obtained with a Rigaku Denki Co. RU-200 diffractometer, using the CuKα radiation at a scanning rate of 1 2θ/min. The voltage and current of the X-ray source were 30 kV and 20 mA, respectively.

The differential scanning calorimetry (DSC) measurements were carried out with a Rigaku Denki, mod. DSC-10 thermoanalytical system, under nitrogen, at a heating rate of 10°C/min. The DSC range and sample weight were 2.5 mcal/s and 2 mg, respectively.

A Rigaku Denki mod. CN-8361 apparatus for thermomechanical analysis (TMA) was used to detect the thermal expansion and contraction properties of the fibers. The heating rate was 10°C/min and dry nitrogen was swept to provide the inert atmosphere. The TMA sensitivity range was ±500 μm/mV.

The surface characteristics were examined with a JEOL JAX-333S scanning electron microscope, at 15 kV acceleration voltage after gold coating.

RESULTS AND DISCUSSION

Amino Acid Composition

The amino acid composition of *A. assama* silk fibroin (Table I) is characterized by the predominance of amino acid residues with small side chains, glycine, alanine, and serine, which total 80.5 mol %. This is a general feature of the fibrous component of many silks.[3] This characteristic primary structure is responsible for the regular secondary structure of silks.[4]

Table I Amino Acid Composition of *A. assama* Silk Fibroin

Amino Acid	Content (mol %)
Asp	5.03
Thr	0.69
Ser	9.16
Glu	1.35
Gly	28.68
Ala	42.62
Val	0.59
Cys	0.34
Met	n.d.
Ile	0.26
Leu	0.33
Tyr	4.86
Phe	0.34
Lys	0.25
His	1.15
Arg	2.53
Pro	0.46
Trp	1.35

Among the other amino acids, tyrosine attains a fairly high concentration (4.9 mol %), similar to other domestic and wild silk fibroins, while acidic and basic amino acid residues account for about 10 mol %.

The family Saturniidae, which includes the silkworm *A. assama,* comprises various Antheraea species, whose chemical composition has been reported by various authors.[1,3,19] The composition of *A. assama* silk fibroin is similar to that of other Antheraea silks, with alanine prevailing over glycine, and a markedly high content of basic and acidic amino acid residues, especially arginine and aspartic acid.

It is interesting to compare the chemical composition of *A. assama* silk with that of the most common tussah silk (*A. pernyi*). *B. mori* silk fibroin is considered as a reference, due to its relevance as a textile fiber and its wide use as a model for studying the relationships between chemical composition and physical properties of fibrous proteins. Table II lists the values of some indexes, which can be useful parameters for inferring physical structure and chemical reactivity of silk fibroins. Both the 100LC/SC ratio, first proposed by Lucas et al.,[1] and the gly/ala ratio can be structural indexes, since they refer to the amino acid residues taking part in the formation of the ordered crystalline regions of the fiber. The P/NP ratio can be related to the chemical reactivity of the fibers, since it evaluates the content of polar amino acids. The latter form the amorphous regions and represent the main reactive sites toward chemicals.

Data reported in Table II confirm that the amino acid composition of *A. assama* and *A. pernyi* are similar. The values of the three parameters are species specific, corresponding to their biological classification. Silks belonging to the family Saturniidae exhibit a gly/ala ratio < 1 and a 100LC/SC ratio larger than *B. mori* silk fibroin. These features, derived from chemical data, correspond to the classification made on the basis of X-ray diffraction patterns.[4,20] In fact, *B. mori* silk fibroin belongs to group 1, while Antheraea species are representative of the group 3a, the main difference being the unit-cell dimensions, which depend on the kind of amino acid residues forming the crystalline polypeptide sequences. The higher value of the P/NP ratio exhibited by Antheraea silks shows that most of the amino acids with long and bulky side chains are polar in character, possessing free amino, carboxyl, and hydroxyl groups potentially reactive toward various chemicals.

Table II Comparative Amino Acid Composition

	A. assama	*A. pernyi*	*B. mori*
GLY + ALA (mol %)	71.30	72.08	74.37
GLY/ALA	0.67	0.71	1.55
P/NP[a]	0.33	0.35	0.27
100LC/SC[b]	23.22	20.35	17.07

[a] Ratio between polar (P) and nonpolar (NP) amino acid residues. P: acidic, basic, and hydroxyl amino acids; NP: other amino acids.

[b] SC = short side chain (alanine, glycine, serine, threonine); LC = long side chain (other amino acids).

Optical Properties

The physical structure of *A. assama* silk fibroin was investigated by measuring refractive indices, from which birefringence (Δn) and isotropic refractive index (n_{ISO}) values were calculated. These are two optical parameters related to the average molecular orientation and crystallinity of the fibers. Δn and n_{ISO} values obtained for *A. assama* silk fibers were 0.030 and 1.557, respectively.

Birefringence is almost similar to that reported for *A. pernyi* (0.034), but significantly lower than that of *B. mori* silk fibers (0.053).[18] The low birefringence is a typical feature of most Saturniidae silks. It has been mainly attributed to the lower amount of laterally ordered regions, a transition phase between crystalline and amorphous regions.[18] In fact, noncrystalline polypeptide sequences of wild silk fibroins would be partially hindered from forming large laterally ordered regions, due to the occurrence of frequent amino acid residues with bulky side chains, which disturb the close packing of adjacent fibroin molecules. The lower degree of order and orientation typical of Saturniidae silks, such as *A. assama* and *A. pernyi,* bears important consequences on the physical properties of the fibers as discussed later.

The isotropic refractive index value is intermediate between those reported for *A. pernyi* and *B. mori* silks (1.542 and 1.559, respectively),[18] suggesting that the crystallinity of the various silk fibroins is not significantly different. However, it should be pointed out that other physical properties, such as fiber density, might exert a noticeable influence on this measure.

Mechanical Properties

The tensile behavior of *A. assama* silk was studied by measuring the stress–strain curves of single fibroin filaments (Fig. 1). These are characterized by

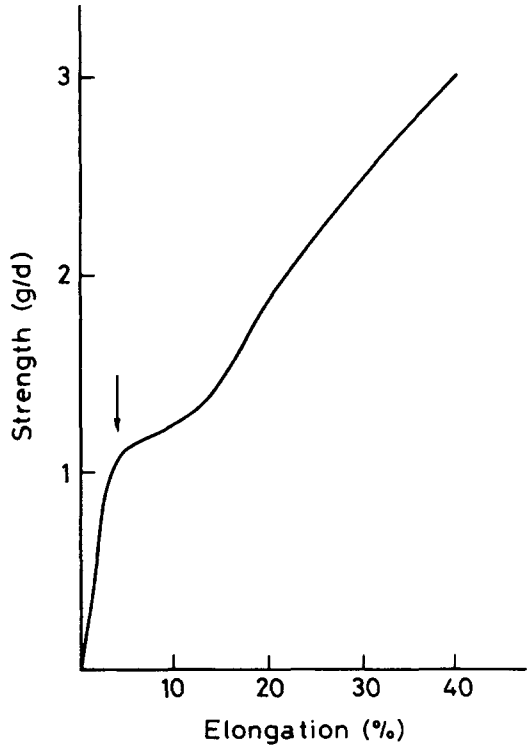

Figure 1 Tensile strength and elongation curve of single *A. assama* fibroin filament. (→) Yield point.

a large elongation at break (about 40%) and by the presence of a marked yield point at an extension of about 5%, followed by a region of gradual extensibility. The mechanical properties of *A. assama* silk fibers are consistent with those reported for other Saturniidae silks.[1] The relatively high degree of extension after the initial resistance to low load has been attributed to unfolding of the long fibroin chains in the amorphous regions.

The tensile properties of *A. assama* silk fibers closely agree with optical data reported in the previous paragraph. It is noteworthy that *B. mori* silk fibers, characterized by a higher degree of order and molecular orientation, do not show a marked yield point in their stress–strain curve. Moreover, they are less extensible and break at higher load.

X-ray Diffraction Curves

Wide-angle X-ray diffraction curves of raw and degummed *A. assama* silk fibers were measured in order to study the crystalline structure (Fig. 2). Raw silk fibers [Fig. 2(a)], taken directly from the cocoon, showed a series of diffraction peaks at scattering angles (2θ) of 16°, 19.8°, 23.8°, and 29.9°, corresponding to crystalline spacings of 5.53, 4.48, 3.73, 2.98 Å, respectively. After degumming, the X-ray diffraction curve [Fig. 2(b)] showed two main peaks at 16.8° and 21.1°, corresponding to the crystalline spacings of 5.27 and 4.41 Å, respectively.

The X-ray diffraction pattern of *A. assama* is typical of silk fibers belonging to the group 3a, according to the classification proposed by Warwicker.[4] It is noteworthy that the group 3a includes most Antheraea species, together with other silks belonging to the family Saturniidae. The antiparallel β-sheet structure is the molecular conformation assumed by the fibroin chains in the crystalline regions, with unit-cell dimensions similar to that of $-(\text{ala})_n-$ in β form.[20]

The minor 2θ peaks of the diffraction curve of raw silk fibers are attributable to calcium oxalate, a typical contaminant of wild cocoons, originating from silkmoth excrements. In fact, the position of these peaks corresponds to the strong reflections of commercial calcium oxalate [Fig. 2(c)]. These results are consistent with the microscopic examinations, showing the presence of numerous foreign particles on cocoon fiber surface.

Thermal Properties

Figure 3 shows the DSC curve of *A. assama* silk fibroin fibers, measured from room temperature to

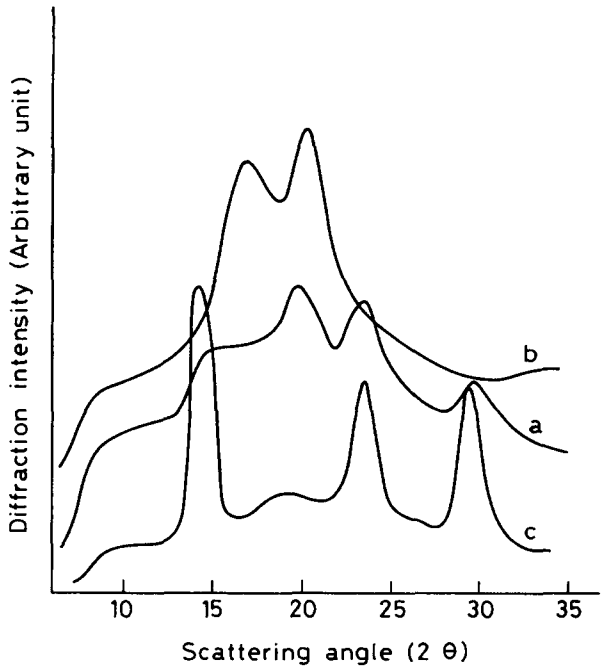

Figure 2 X-ray diffraction intensity curves of (a) *A. assama* cocoon shell, (b) *A. assama* fibroin fibers, and (c) commercial calcium oxalate.

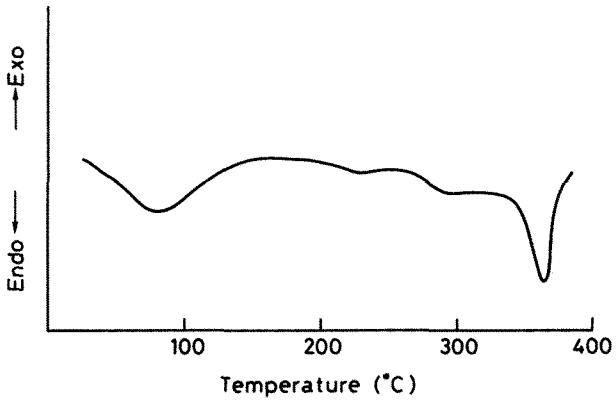

Figure 3 DSC curve of *A. assama* fibroin fibers.

400°C. The first broad endotherm below 100°C is due to the evaporation of water. As the temperature increased, the fiber showed noticeable thermal stability, until above 200°C. Two minor and broad endothermic transitions appeared at 230 and 300°C (shoulder form), followed by a prominent endothermic peak at 362°C. The latter is attributable to the thermal decomposition of silk fibers with β conformation.[21] The two minor endotherms occurring above T_g (190–200°C)[22] can be related to the molecular motion of the fibroin chains either in the amorphous and laterally ordered regions[15] or in the crystalline regions, because the spacings corresponding to the intersheet distance of tussah silk fibers gradually expand at above 190°C.[23]

The thermal behavior of *A. assama* silk fibers was further investigated by measuring the expansion and contraction properties in the course of heating (Fig. 4). The TMA curve showed a constant drop from room temperature to about 160°C, suggesting a rubber elastic behavior of oriented noncrystalline molecules. The extent of contraction attained was about 2%, a value intermediate between those reported for *B. mori* (0.7%) and *A. pernyi* (3.6%) silk fibers.[18] The length of the sample remained unchanged until 180°C, when it began to extend slightly. The final abrupt extension occurred at above 220°C, as a consequence of the breaking of interchain linkages induced by heating.

The thermomechanical behavior of *A. assama* silk fibers in the temperature range below T_g is consistent with the fine structure elucidated by optical (birefringence) and mechanical properties, that is, the disordered arrangement of the fibroin chains within the amorphous and laterally ordered regions. However, ultrastructural features, such as fiber porosity, may influence thermomechanical measurements. Since it has been reported that several vacuolar droplets of different shape and size are present in tussah silk filaments,[24] the difference in the extent of contraction between *A. assama* and *A. pernyi* silk fibers might be related to a slightly less porous structure. This hypothesis is consistent with the high value of isotropic refractive index measured on *A. assama* fibers, which may account for a denser and more compact fibrous texture, rather than a higher degree of crystallinity.

Surface Characteristics

Surface characteristics of *A. assama* silk fibers were studied by scanning electron microscopy. Figure 5(a) shows a longitudinal view of raw silk fibers taken from the cocoon. A large number of regular crystals are deposited on the fiber surface. These are calcium oxalate deposits, left by the silkworm during spinning (excrements).

Degummed fibers [Fig. 5(b)] are almost completely free of foreign deposits and show the presence of distinct longitudinal striations, with a thickness of 0.4–1 μm, running parallel along the fiber axis. This characteristic is common to other wild silk filaments, bearing a striking contrast with the very smooth appearance of *B. mori* silk, and is also responsible for the lower degree of luster of textile products made by wild silk.

Fiber cross sections are variously shaped [Fig. 5(c) and (d)], the elliptical shape being prevalent. Moreover, some cross sections are very flat, appearing as a ribbon. Voids are visible within the cross section of individual silk filaments. It is worth mentioning that the average filament size is twice than that of *B. mori*.[19]

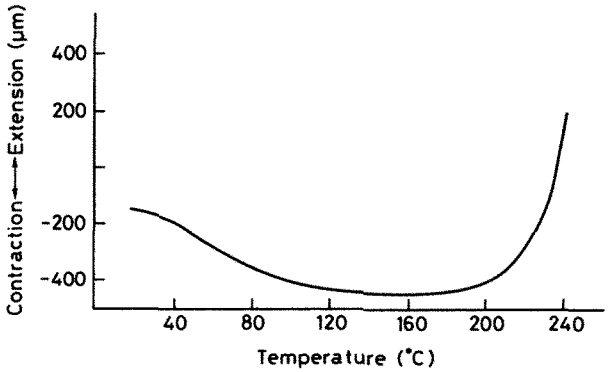

Figure 4 TMA curve of *A. assama* fibroin fibers.

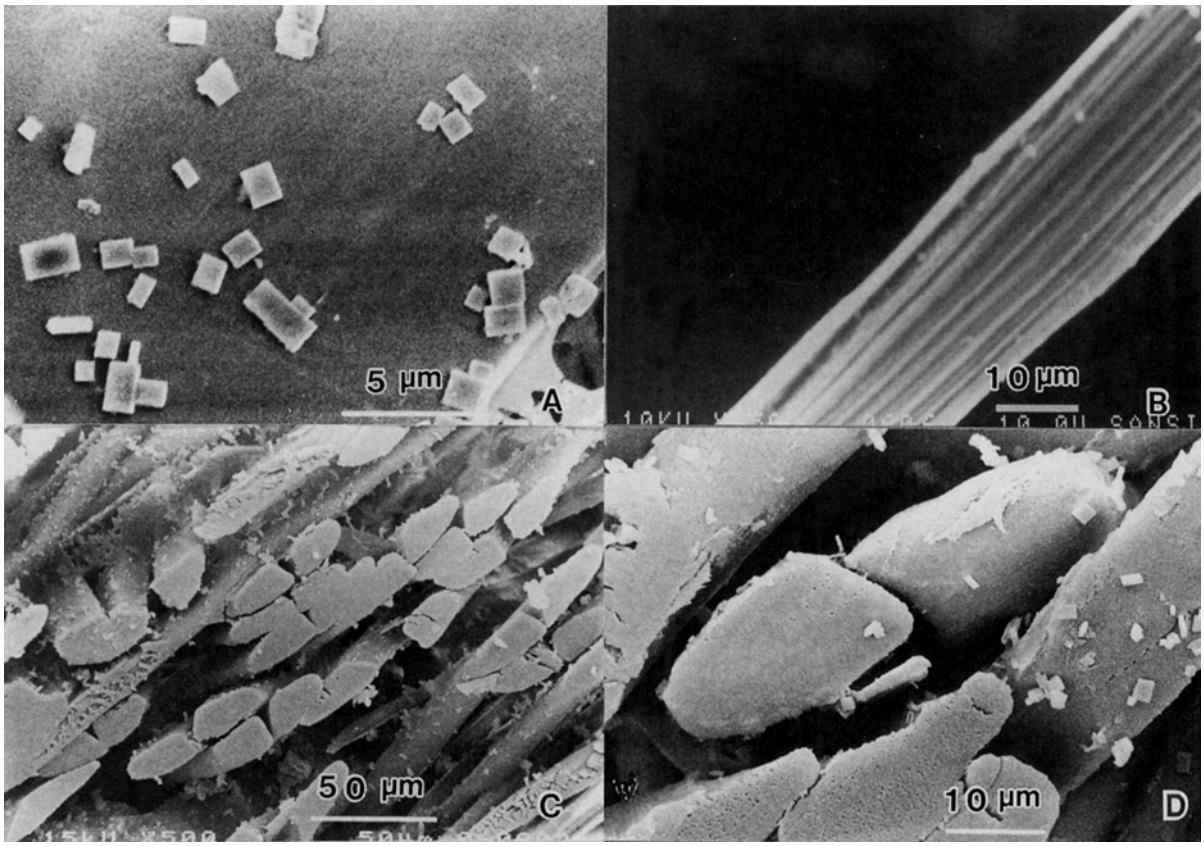

Figure 5 SEM photographs of *A. assama* silk. (a) cocoon shell with calcium oxalate crystals, (b) longitudinal view of degummed silk fibers, (c) and (d) cross sections of fibroin fibers.

CONCLUSIONS

Silk fibers produced by the silkworm *A. assama* showed a striking resemblance to other Antheraea silks. Chemical indexes, calculated on the basis of the amino acid composition, are consistent with the biological classification. X-ray diffraction results confirm that this silk belongs to the X-ray group 3a.[20] Both birefringence and tensile behavior are indicative of a low degree of order and molecular orientation of the fibers. The most interesting feature of *A. assama* silk fibers, compared to *A. pernyi*, is the slightly higher thermal stability emerging from TMA data. This characteristic has been attributed to the lower porosity of the fibrous texture, in good agreement with the markedly high value of isotropic refractive index, which seems to suggest a denser fiber structure.

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