THE FINE STRUCTURE OF SILK FIBROIN

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ABSTRACT

The fine structure of *Bombyx mori* silk fibroin was investigated by electron microscopy and X-ray diffraction techniques. Examination of silk fibers fragmented with ultrasonic radiation and negatively stained revealed the presence of ribbon-like filaments of well-defined lateral dimensions. Analysis of the breadths of the equatorial reflections in the X-ray diffraction pattern of fibroin yielded similar dimensions for the lateral extent of the crystallites. It is concluded that the crystalline material in *B. mori* silk fibroin is in the form of ribbon-like filaments of considerable length parallel to the fiber axis and of lateral dimensions approximately 20 × 60 A.

INTRODUCTION

There is abundant evidence that the cocoon fibers produced by the larvae of the silk moth *Bombyx mori* have a filamentous fine structure (14, 16, 20), but no clearly defined microfibril of the type encountered in other fibrous proteins such as feather keratin (4) and mammalian keratin (1, 17) has been recognized so far. Early studies of *B. mori* silk fibroin by X-ray diffraction techniques (2, 9) revealed that it contained crystallites which were elongated parallel to the fiber axis but of limited extent or poor regularity at right angles to this direction. Kratky and coworkers (9, 10) showed that mechanical deformation of native silk glands produced double orientation, which suggested that the lateral dimensions of the crystallites were unequal, and this was later confirmed by studies of stretched and rolled silk fibroin (11).

The present communication describes a study of the filamentous fine structure of silk fibroin and its relationship to the crystallites revealed by X-ray diffraction studies. The results obtained suggest that there is a ribbon-like microfibril of well-defined dimensions in *B. mori* and other silk fibroins.

MATERIALS AND METHODS

Electron Microscopy

Degummed silk fibers were cut into short lengths and irradiated in 98–100% formic acid in an ice-cooled dispersion cell of an M.S.E. (Measuring Scientific Equipment, England) ultrasonic generator (rated at 60 watts, 20 kc/sec) for 4 hr. In addition to swelling the silk, the formic acid provided a convenient dispersion medium which could be removed by repeated dilution with distilled water. After irradiation, the contents of the cell were centrifuged at 600 g for 15 min, and the supernatant liquid was discarded. The debris was washed three times with water and finally redispersed by a further brief irradiation. A drop of the suspension was deposited onto a carbon-collodion-supporting film by means of a platinum loop and was allowed to dry.

Solubilized silk was prepared by treating degummed silk with cupriethylene diamine following the method described by Drucker, Hainsworth, and Smith (3). The gelatinous solution obtained after dialysis was suitably diluted and a drop deposited on a grid. Samples of the synthetic polypeptide poly-L-alanylglycyl - L - alanylglycyl - L - serylglycine, which was synthesized as a model of the crystalline portions
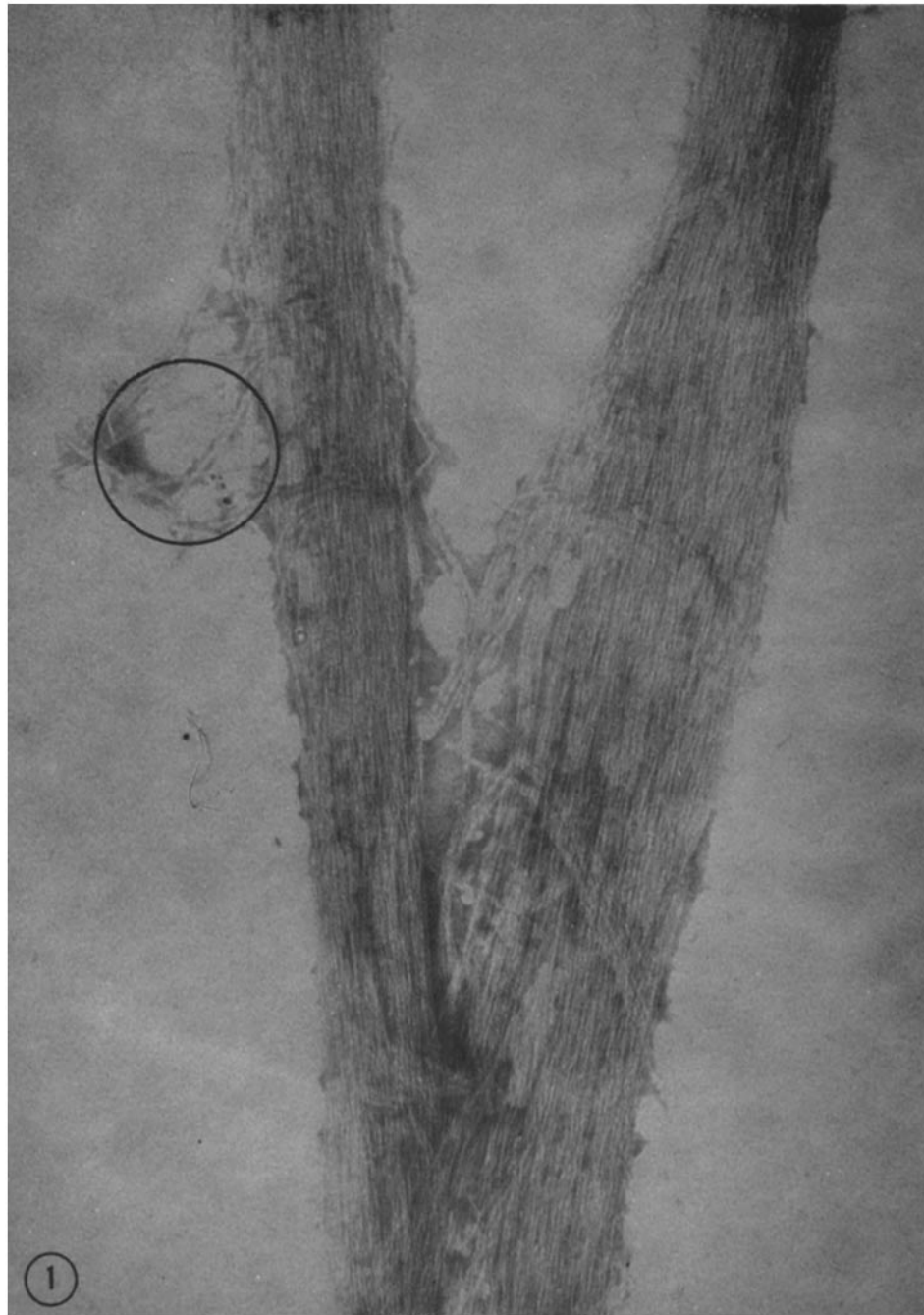
FIGURE 1   Disintegrated *Bombyx mori* silk negatively stained with sodium phosphotungstate, showing sheets of filaments. ✕ 120,000.

of *B. mori* fibroin (19), were prepared from a 0.1% solution of the polymer in formic acid by depositing a drop of the solution on a grid.

In each case the specimens were negatively stained with 1% sodium phosphotungstate at pH 5.6 and examined in a Siemens Elmiskop I operating at a potential of 80 kv and employing the double condenser system; 50-μ objective apertures were used throughout.

X-Ray Studies

High-angle X-ray diffraction patterns were recorded by a 3-cm cylindrical camera operated *in vacuo*, and low-angle patterns were recorded by an evacuated 5-cm flat film camera with 50-μ collimation. The intensity profiles of the high-angle equatorial reflections were measured with a Joyce-Loebl microphotometer and the variation of intensity (I)
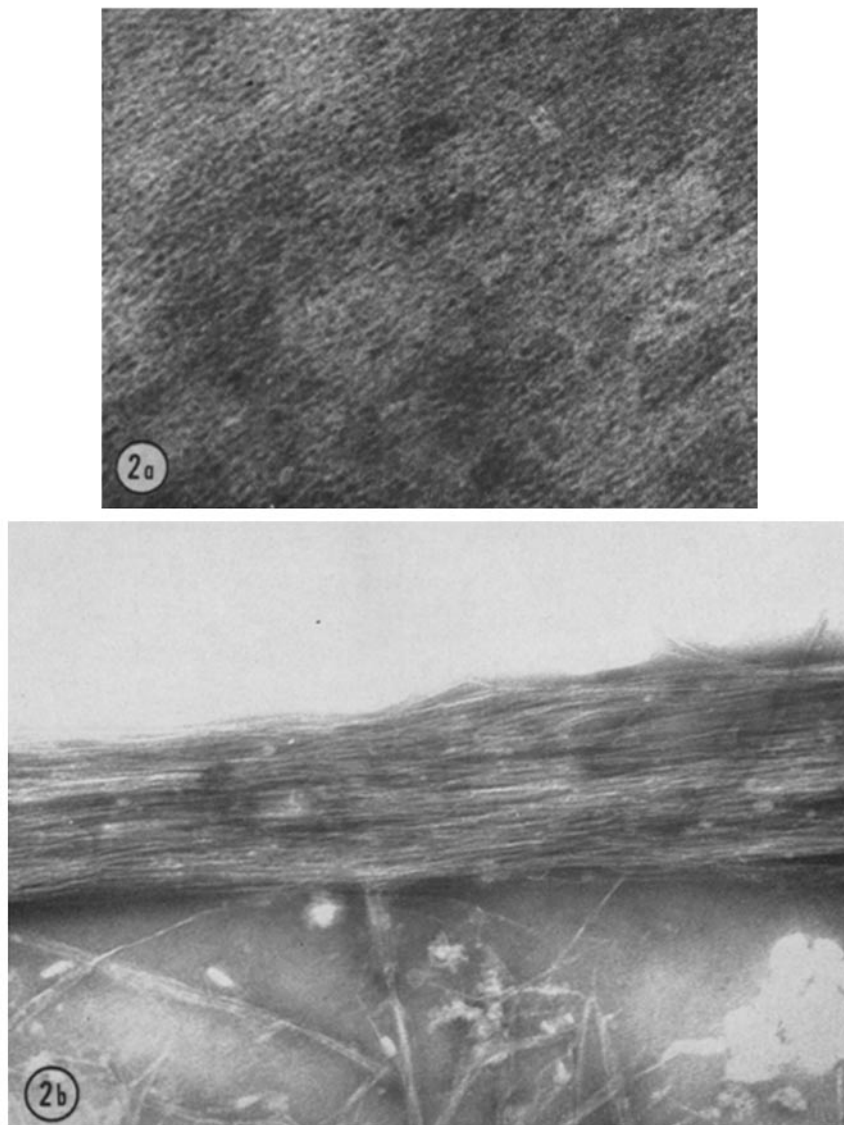

Figure 2 *a*   Transverse section of osmium tetroxide-stained *Bombyx mori* silk showing a layered organization of filaments. × 250,000.

Figure 2 *b*   Sheet of filaments obtained from *Antherea pernyi* silk, negatively stained with sodium phosphotungstate. × 120,000.

with reciprocal space coordinate (R) analyzed into a series of component Cauchy curves having the general formula

$$I = I_{max}/\{1 + [2(R_{max} - R)/\Delta R]^2\},$$

where R_{max} and I_{max} specify the position and intensity of the peak and ΔR its half width. The analysis, with a least squares procedure being used (5), was performed on a CDC 3600 computer. The reciprocal of the half width of each component was then taken as an indication of the size of the crystallite in a direction at right angles to the set of planes responsible for the particular reflection (8).

RESULTS AND DISCUSSION

Bombyx mori Fibroin

The debris obtained by ultrasonic disintegration of fibroin consisted of thin sheets of material as illustrated in Fig. 1. These sheets appear to consist of filaments about 20–25 A wide, in the plane of the section, separated by 15–20 A of a more densely stained material. In a number of instances, mostly near the edges of sheets, examples of filaments 55–65 A wide were observed; a typical case is shown in the circle in Fig. 1. These observations suggest that silk contains ribbon-like filaments of well-defined lateral dimensions which may conveniently be referred to as microfibrils. Early studies of silk revealed the presence of filamentous material, and estimates of their lateral dimension have ranged from 40–300 A (18). In particular, Zahn (20) suggested that the filamentous material consisted of microfibrils 40–80 A wide. In most instances the methods of preparation or examination would have precluded the recognition of a highly asymmetric cross-sectional shape in the microfibril. This asymmetric shape is confirmed by the cross-section shown in Fig. 2 a where the dimensions of the unstained areas agree well with those deduced from Fig. 1. With microfibrils of such small dimensions the interpretation of the appearance in cross-section is complicated by the possibility that the plane of the section is not accurately normal to the axis, but the packing of the microfibrils in Fig. 2 a appears to be irregular, apart from a marked tendency for the planes of the ribbons to lie parallel. This explains how it is possible to obtain fragments of the type shown in Fig. 1 in which the majority of the ribbons present their narrow aspect to the electron beam. It follows also that the microfibrils adhere more strongly via the larger surface since fragmentation

apparently proceeds most readily along lines perpendicular to the larger lateral dimension.

Current ideas of the arrangement of the polypeptide chains in silk fibroin are based on the proposal of Marsh, Pauling, and Corey (14) that the chains crystallize in an antiparallel-chain pleated-sheet conformation with one surface of each sheet entirely populated by glycyl residues. The sheets are supposed to be arranged in pairs with their glycyl surfaces in contact, giving an alternation in intersheet spacing. The unit cell of this proposed structure contains four chains and has lateral dimensions $a = 9.4$ A in the plane of the hydrogen-bonded sheet and $c = 9.2$ A perpendicular to this plane. The equatorial X-ray reflections arising from planes normal to c are more diffuse than from those from planes normal to a, a fact which indicates that the crystallite is smaller or less perfect in a direction parallel to the c axis, that is, normal to the pleated sheets. The analysis of the profiles of these reflections is summarized in Table I, together with the lateral dimensions of the crystallites d_a, parallel to the a axis, and d_c, parallel to the c axis, deduced from the breadths of the reflections.

The close correspondence between d_a and d_c and the lateral dimensions of the microfibrils strongly suggests that the microfibrils constitute the crystalline material. If this is so, it follows that the hydro-

TABLE I

The Lateral Dimensions of the Crystallites in B. mori Fibroin Estimated from the X-ray Diffraction Pattern

Reflection	Half width	d_a*	d_c
	A^{-1}	A	A
001	0.0425	—	24
002	0.048	—	21
003	0.043	—	23
201‡	0.024	—	19
400	0.018	56	—
Origin peak§	—	62	—
Mean value		59	22

* d_a is the estimated dimension in the plane of the pleated sheets; d_c is the estimated dimension normal to the sheets.
‡ The normal to the 201 planes is inclined at 27° to the a axis, hence $d_c = \sin 27°/$half width.
§ Estimate obtained by assuming the low-angle maximum at 43.5 A is the first subsidiary maximum of a crystallite of rectangular cross-section.
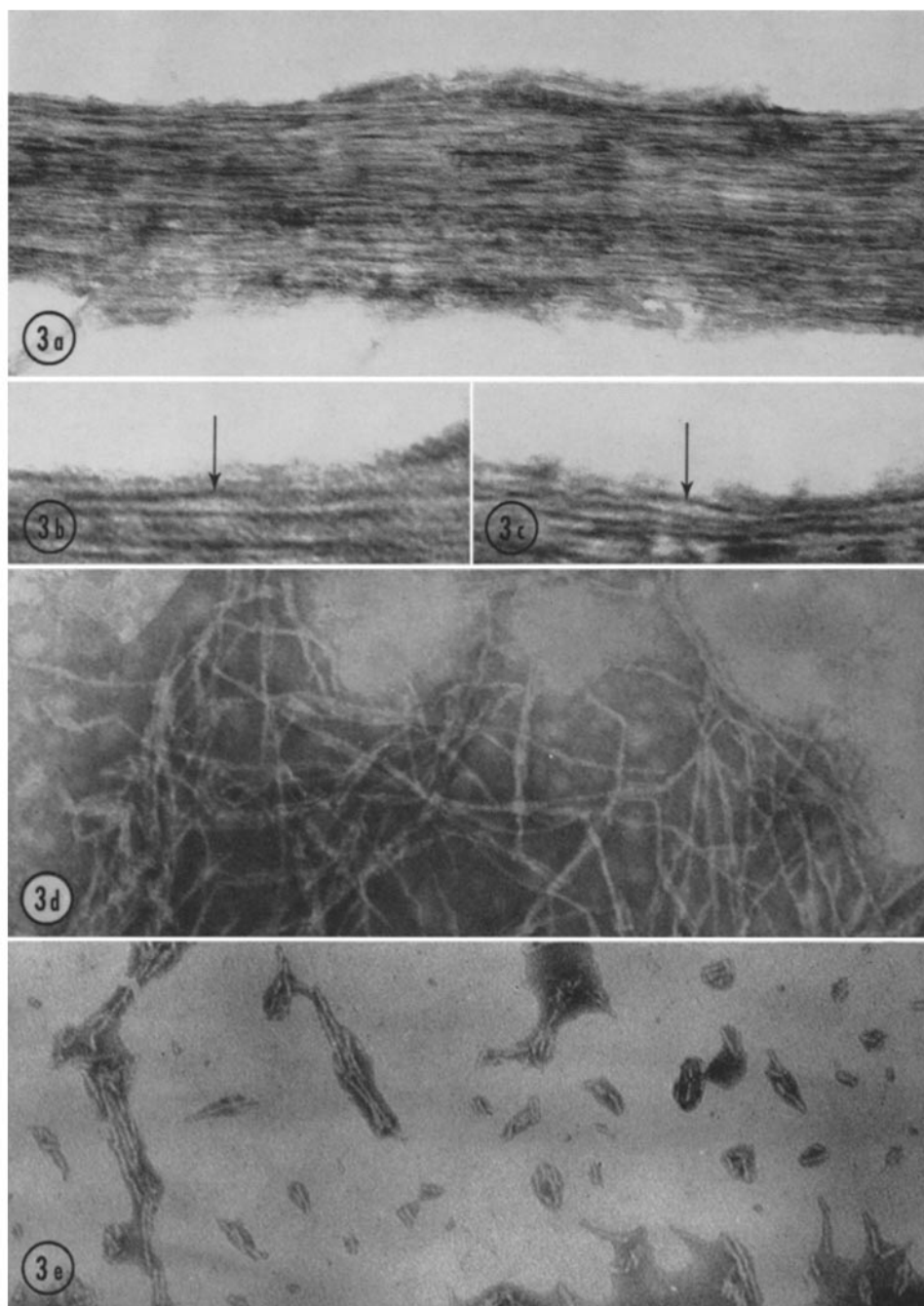
FIGURE 3 a Disintegrated *Anaphe moloneyi* silk negatively stained with sodium phosphotungstate, showing sheets of filaments. $\times$ 120,000.

FIGURES 3 b and 3 c As for Fig. 3 a, but showing filaments twisting through 90° and presenting their wider aspect towards the electron beam. $\times$ 350,000.

FIGURE 3 d Film cast from a solution of *Bombyx mori* fibroin, obtained by treatment with cupriethylene diamine, negatively stained with sodium phosphotungstate. $\times$ 120,000.

FIGURE 3 e Filaments obtained from the sequential polypeptide (ala-gly-ala-gly-ser-gly)$_n$, negatively stained with sodium phosphotungstate. $\times$ 120,000.

gen-bonded pleated sheets must lie parallel to the longer lateral dimension of the microfibril.

According to Lucas, Shaw, and Smith (13), about 60% of the polypeptide chain in silk consists of segments having the sequential formula gly-ala-gly - ala - gly [ser - gly - (ala - gly)$_n$]$_8$ - ser - gly-ala-ala-gly-tyr, where n has a mean value of 2, and it has been assumed that these segments are derived from the crystalline regions of the fiber. If this is so, it follows that all the charged and bulky residues must be incorporated in either less crystalline or amorphous regions of the structure and may account for the intermicrofibrillar material seen in Figs. 1 and 2. A lateral alternation of crystalline and amorphous material of this type is favored by Zahn (20) and would account for such properties as the anisotropic swelling during water sorption (7).

Anaphe moloneyi and Antherea pernyi Fibroins

The amino acid compositions of different moth silks show considerable variations from species to species (13), and it was of interest to examine samples of two silks markedly different in composition from B. mori silk. The appearance of the debris obtained by ultrasonic disintegration of these silks is shown in Figs. 2 b and 3 a, and in both cases will be seen to be very similar to that obtained from B. mori fibroin. Two examples are shown in Fig. 3 b and c of Anaphe silk debris in which a filament 20–25 A wide broadens gradually to about 60 A and then returns to its original width. These are believed to represent instances where the ribbon-shaped microfibril is twisted through about 90° over part of its length, so presenting its broader aspect.

X-ray diffraction patterns obtained from the Anaphe silk were not of such high quality as those form B. mori silk, but it was possible to obtain an estimate of d_c which proved to be very similar to that deduced for B. mori silk. The half widths of the equatorial reflections of the Antherea silk are very similar to those of B. mori silk (15).

Soluble Fibroin and Sequential Polymer

Both solubilized fibroin and the model polypeptide poly - L - alanylglycyl - L - alanylglycyl - L-serylglycine are known to crystallize in the same conformation as the polypeptide chains in the crystallites of native silk fibroin (6), and it was found that films cast from solutions of these materials consisted of filamentous material as shown in Fig. 3 d and e. The filaments obtained from solubilized fibroin are variable in length and show numerous kinks. They are extremely fine, being only about 20 A in diameter, and appear to aggregate by twisting around each other. The filaments observed in the synthetic polypeptide are again of the order of 20 A wide but much shorter than in the natural material. The polypeptide contains only glycyl, alanyl, and seryl residues, and this may explain why there is less tendency for the molecules to aggregate longitudinally.

Correlation with Solution Studies

Recent studies (12) of the scattering of X-rays by solutions of B. mori fibroin obtained from the spinning glands of the silkworm have been interpreted in terms of a dispersion of elongated particles which presumably aggregate end-to-end to form the microfibrils observed in silk. The dimensions of a particle considered as an elliptical cylinder were 212 × 66 × 19 A. If, as seems likely, end-to-end aggregation occurs via the longest dimension, the two lateral dimensions of 66 and 19 A would correlate well with the lateral dimensions of the microfibrils deduced from the present electron micrograph and X-ray studies.

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