Electron Diffraction Studies on Indian Silk*

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Synopsis

The lateral order factor of four Indian varieties of silk, viz., Mulberry, Tasar, Eri, and Muga, were determined by electron diffraction technique and compared with that determined by x-ray diffraction. The profiles of the 002 and 201 reflections in Mulberry were better resolved by the electron diffraction technique.

INTRODUCTION

Structure investigation of silk fibroin has been carried out by many workers using different techniques such as x-ray diffraction,[1,2] infrared spectroscopy,[3] amino acid analysis,[4] and electron microscopy.[5,6] Detailed x-ray diffraction studies by Marsh et al.[1,7] and Warwicker[2] have established that fibroin has a parallel β-type structure. The unit cell proposed by these workers is orthorhombic. Magoshi et al.[8,9] have studied the α–β transition of regenerated silk obtained from the glands of mature silkworms. Recently, studies have been made on synthetic poly(α-amino acids) using x-ray and electron diffraction techniques[10,11] to elucidate elaborately with models the structure of silk fibroin. However, electron diffraction has not been used extensively for structure investigation of the silk fibroin. In this paper, we report the electron diffraction results obtained for different varieties of Indian silk.

EXPERIMENTAL

Materials

Mulberry (*Bombyx mori*), Tasar or Tussah of India (*Antheraea mylitta*), Eri (*Philosamia cynthia ricini*), and Muga (*A. assamensis*) silks were used for the studies. Mulberry silk yarn was degummed by treating with textile soap at 100°C for 1 hr with a material:liquor ratio of 1:50. The sample was washed with hot and cold water and finally dried under vacuum. The wild silk yarns were degummed by treating with textile soap in the presence of washing soda (0.5 g/l.) at 100°C for 1 hr with a material:liquor ratio of 1:50. The sample was rinsed with hot and cold water. The degumming was repeated.[12]

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Equipment and Procedure

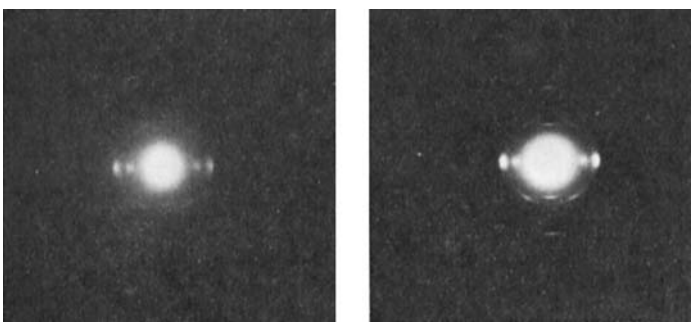
The electron diffraction technique employed was essentially the same as that described by Paralikar and Betrabet.[13] The silk filaments were thoroughly beaten in a high-speed laboratory blender. A drop of diluted slurry of silk fragment was placed on uncoated 400-mesh copper grid and dried at room temperature. A Hitachi HU 11E electron microscope was used. The experimental conditions were, in brief: use of liquid nitrogen throughout the experiment to cool the specimen, accelerating potential of 75 kV with extremely low-beam current, and exposure time of 5 sec. Before examining the silk specimen, a grid supporting thin film of Al was inserted in the microscope and the microscope was preset in the diffraction mode and focused to get a sharp typical Al pattern. This served two purposes. The camera constant could be determined and scanning of grids supporting silk specimen in the bright-field transmission mode was avoided, thereby minimizing to the utmost any degradation owing to the electron beam. Then grid supporting the Al film was replaced by the grid supporting the silk fragment, and a selected area diffraction pattern of silk fibroin formed at the back focal plane of the objective lens and magnified by appropriate lenses was recorded on a Fuji orthochromatic electron microscope film. The conditions of developing and fixing the film were the same as those described in an earlier publication.[13]

RESULTS AND DISCUSSION

Electron Diffraction Patterns

The electron diffraction patterns for Mulberry and Tasar silk are shown in Figure 1. The unit cell parameters proposed by Marsh et al.[1,7] were used to determine the *hkl* values for different equatorial reflections. The electron diffraction pattern for Mulberry shows reflections corresponding to indices 002, 201, 300, and 003. Since the electron diffraction patterns for Tasar, Eri, and Muga are similar, the pattern for Tasar alone is reproduced here; the reflections correspond to indices 002, 201, 003, and 300.

Table I summarizes the interplanar spacings for equatorial reflections of the four varieties of Indian silk observed by electron and x-ray diffraction[19] tech-



(a) (b)

Fig. 1. Electron diffraction pattern of cooled fragment of silk taken at 75 kV and exposure time of 5 sec: (a) Mulberry; (b) Tasar.

TABLE I
Interplanar Spacings for Equatorial Reflections of Different Varieties of Indian Silk Observed by Electron and X-Ray Diffraction Techniques

Variety of silk	*hkl*	*d* Spacing, Å		Calculated *d* spacing, Å
		Electron diffraction	X-ray diffraction[19]	
Mulberry	002	4.78	4.70	4.72
	201	4.27	4.35	4.19
	300	3.29	3.28	3.15
	003	3.16	3.18	3.07
Tasar	002	5.33	5.28	5.30
	201	4.68	4.45	4.28
	003	3.62	3.71	3.53
	300	3.18	3.21	3.13
Eri	002	5.20	5.35	5.30
	201	4.57	4.33	4.28
	003	3.66	3.71	3.53
	300	3.18	3.20	3.13
Muga	002	5.31	5.27	5.30
	201	4.58	4.33	4.28
	003	3.65	3.70	3.53
	300	3.15	3.18	3.13

niques. It may be noted that most of the lattice spacing values determined from the reflections obtained by the electron diffraction technique are in good agreement with those calculated theoretically. However, in the case of wild silks, the *d* spacing corresponding to the 201 plane is a little on the higher side.

Further, it is noted that the positions of the 300 and 003 reflections in the case of Mulberry are interchanged when compared with the wild silks, because of the basic difference in their unit cell parameters with respect to the *c* axis. In the case of Mulberry, the value for *c* is 9.2 Å; and in wild silks, $c = 10.6$ Å.

Equatorial Tracings

The electron diffraction patterns for Mulberry, Tasar, Eri, and Muga were equatorially scanned using a sensitive microphotometer. A correction for background scattering and other extraneous scattering was applied to the integrated intensity curves.[14] Figure 2 illustrates the corrected equatorial intensity curves for all four varieties of silk. The lateral order factor was calculated for each sample based on the procedure proposed by Manjunath et al.[15] for x-ray diffractograms of polymer, viz.,

$$R_f = \frac{m_1 + 2m_2 + m_3 \cdots m_{n-1}}{h_1 + h_2 + h_3 + \cdots h_n}$$

$$L_o = (1 - R_f)$$

where R_f is the resolution factor, L_o is the lateral order factor; $h_1, h_2, \cdots h_n$ are the respective peak heights, and $m_1, m_2, \cdots m_{n-1}$ are the respective minimum heights between the two peaks representing amorphous contribution. The R_f is inversely related to the lateral order, i.e., R tends to be 1 when the resolution is completely lost and it tends to be zero when the resolution is maximum.

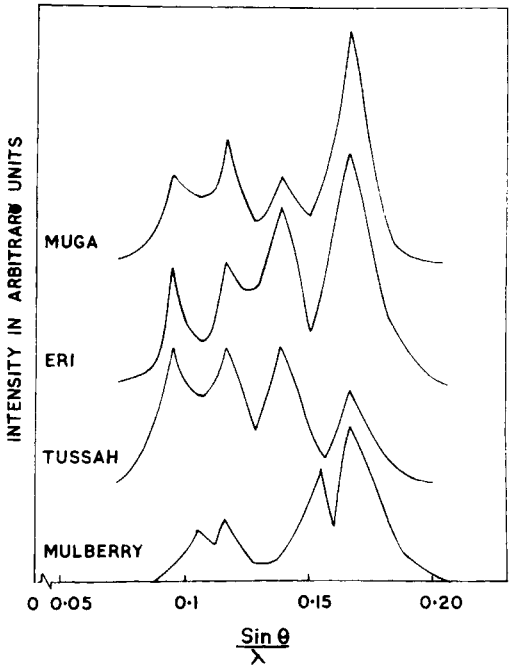


Fig. 2. Intensity tracings along the equator of the electron diffraction patterns of four varieties of silk. The reflection profiles correspond to planes 002, 201, 003, and 300 for three wild silks and planes 002, 201, 300, and 003 for Mulberry.

Lateral Order Factor

It is observed that the L_o factor calculated using the above formula could be related directly to the crystallinity index. Table II shows the values of order factor obtained using electron diffraction and x-ray diffraction[19] techniques.

The L_o of cotton determined by the electron diffraction technique is 0.74 (unpublished data). As compared to this value, the L_o factor of silks obtained by either x-ray diffraction or electron diffraction seems to be low. However, such a low value can be accounted for if one considers carefully the bulky side groups occurring in the polypeptide chain. It is believed that only simple amino acids such as glycine, alanine, and serine make up the ordered region in the silk fibroin. Since the bulky amino acids such as tyrosine, tryptophan, and phenylalanine cannot be accommodated in the regular array, they make up most of the amorphous part.

TABLE II
Lateral Order Factor for Different Varieties of Indian Silk

Variety of silk	Order factor by electron diffraction technique	Order factor by x-ray diffraction technique[19]
Mulberry	0.62	0.42
Tasar	0.60	0.43
Eri	0.62	0.44
Muga	0.63	0.43

The crystalline fraction of the silk fibroin has been separated by enzymolysis,[16] alkali hydrolysis, acid hydrolysis, and oxidative degradation.[17] Warwicker[18] has shown the residue obtained by partial acid hydrolysis as having crystalline structure identical to the crystalline structure determined for silk yarns. Furthermore, the amino acid composition for these residues is shown to be mostly due to simple amino acids such as glycine, alanine, and serine.[17] The studies of Zuber[20] and Shaw[21] on various fractions obtained from silk fibroin after chymotryptic and tryptic digestion has revealed that fibroin consists of three phases. The sequences of glycine, alanine, and serine give rise to phase I, which is highly crystalline and gives rise to a characteristic x-ray pattern. Phase II is mainly due to glycine, alanine, valine, and tyrosine, which is a mixture of sequences of phase I and other sequences. Phase II is probably imperfectly ordered and randomly oriented. Lastly, phase III consists of the remaining amino acid sequences consisting mainly of polar and high molecular weight residues. Of 100 amino acid residues in fibroin, approximately 61 occur in phase I, 30 in phase II, and 9 in phase III. On this basis, one can expect a 60–90% crystallinity for silk fibroin. However, because of conformation defects, the ideal conditions are not fulfilled, and as a result, the lateral order in the silk fibroin may be reduced somewhat. Furthermore, the voids in the fibroin may give rise to diffuse scattering and lower the resolution.

With this in view, the order factor obtained from x-ray studies (0.43) seems not to be in conformity with the amino acid analysis. However, the results obtained by electron diffraction studies yield an order factor of about 0.60, which is in good agreement with the phase concept of Zuber[20] and Shaw.[21] Further, it can be recalled that the percent crystallinity calculated by Badger et al.[22] for *Bombyx mori* silk using infrared spectroscopy was 63–67%. This seems to be in agreement with the value for the percent crystallinity reported using a chemical method.[16] The marked difference in the values of order factor as determined by x-ray diffraction and electron diffraction is largely due to the differences in the two techniques. It may be noted that the electron diffraction result is obtained from a fragment (consisting of tiny mosaic crystals) with a very small area under the electron beam.

Further, the electron diffraction technique seems to be more sensitive and to have certain advantages over the x-ray diffraction technique. This is supported by the observation with respect to the 002 and 201 reflections in the case of Mulberry. These reflections could not be resolved properly in the x-ray studies[1,19] but could be resolved well in the present study by the electron diffraction technique (Fig. 2). The advantages of the selected area electron diffraction technique in obtaining more information on the unit cell geometries and parameters of model synthetic polypeptides have also been highlighted by Lotz et al.[11] Our studies based on the fragments of silk of different varieties are in good agreement with the results of Lotz et al.[11]

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