Crystal size distribution in pure Nistari silk fibres

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Wide-angle X-ray diffraction studies of pure Nistari silk fibres and also fibres annealed at various temperatures for different periods of time were carried out to evaluate crystal size and lattice distortion parameters, as these determine the properties of silk fibres.

(Keywords: silk fibres; crystal size distribution; wide-angle X-ray diffraction)

INTRODUCTION

Silk, which is a fibrous protein, is one of the industrially important fibres. Wide-angle X-ray scattering (WAXS) studies of silk fibres by earlier investigators have shown that they are partially crystalline[1]. For a perfect crystal the diffraction pattern would comprise an array of very small spots. For silk fibres the reflections are broader (owing to finite crystallite size and lattice distortion) and arced (owing to misorientation effects)[2]. Johnson's group at Leeds, UK[3], have reported in their paper an elegant profile separation method using Cauchy and Gaussian distributions. They have also compared the parameters obtained by integral breadth (non-transform) and Fourier analysis (transform) methods, when two orders of X-ray reflections are present. Using Fourier analysis of the scattered X-ray reflections we have attempted in this paper to determine crystal size and lattice distortion parameters for two equatorial reflections of pure Nistari silk fibres, and also we have studied the effect of annealing on these parameters. We would like to emphasize that such studies for pure Nistari silk fibres have not been reported earlier. The cell parameters of silk fibres have been reported by earlier investigators[4–6].

Both multiple- and single-order methods used to separate crystal size and distortion parameters are derived from the theory of Warren and Averbach[7] utilizing the Fourier cosine coefficients of the intensity profile. Somashekar *et al.*[8] and Hall and Somashekar[9] have considered various aspects of multiple- and single-order methods. Recently we have extended the single profile method to natural polymers[10].

THEORY

The intensity profile of X-ray reflection from a partially crystalline fibre is a function of the distribution of crystal size $\langle D\rangle$ and lattice distortion g. These are related through the Fourier coefficients $A(n)$ to the profile intensity $I(s)$ by the equation:

$$I(s)=\sum_{n=-\infty}^{\infty} A(n)\cos[2\pi nd(s-s_0)] \qquad (1)$$

Here s_0 is the value of $s=(\sin\theta/\lambda)$ at the peak of the profile, d is the mean d-spacing of the lattice causing the reflection and n is the harmonic number. The Fourier coefficients can be factorized into size $A_s(n)$ and disorder $A_d(n)$ coefficients as:

$$A(n)=A_s(n)A_d(n) \qquad (2)$$

These are not normalized. By taking an exponential distribution function for crystal sizes we have the following relations for $A_s(n)$:

$$\begin{aligned} A_s(n)&=A(0)(1-n/\langle N\rangle) && n\leqslant p \\ A_s(n)&=A(0)\exp[-\alpha(n-p)]/\langle N\rangle && n\geqslant p \end{aligned} \qquad (3)$$

where $\langle N\rangle$ is the average number of unit cells in a column through the crystal direction normal to the lattice planes causing the reflections. Here p is the smallest number of unit cells in a column and $\alpha=1/(\langle N\rangle-p)$ is the width of the distribution function of column lengths. The crystal size is given by:

$$\langle D\rangle=\langle N\rangle d_{hkl} \qquad (4)$$

and $A_d(n)$ is the disorder coefficient for a paracrystal with separation of neighbouring lattice planes having a Gaussian distribution of standard deviation given by:

$$A_d(n)=\exp(-2\pi^2m^2ng^2) \qquad (5)$$

where m is the order of the reflection and g is the lattice distortion parameter.

The reason for using non-normalized Fourier coefficients are (i) truncation of the profile and (ii) error in the background estimation. These affect the low-order Fourier coefficients of the intensity profile as explained in detail by Somashekar *et al.*[8]. We have therefore used an asymmetric exponential distribution function for the

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crystal size distribution, which gives fairly good results. Using equations (1), (2), (3) and (5) along with the experimental intensity data, it is possible to obtain both crystal size and lattice distortion.

EXPERIMENTAL AND COMPUTATION

Nistari silk fibre is from the multivoltine race of mulberry silkworm (*Bombyx mori*). The cocoons are kept in boiling water for 3–4 min and the fibre reeling is processed at 45°C. Samples of Nistari fibres were annealed at 100, 140 and 200°C for various lengths of time without stretching the fibre.

The X-ray diffraction profile of equatorial reflections from silk fibres, recorded using an X-ray diffractometer (JEOL, Japan, target Fe, $\lambda = 1.934$ Å), is given in *Figure 1* and shows only two reflections, (1 0 0) and (2 1 0). These reflections were assumed to be symmetric, and the half where the overlap with the neighbouring reflections is minimum was used to determine the Fourier coefficients $A(n)$. In an earlier paper[8], it has been reported that using a variable background[11] evaluated by fitting a Fourier Bessel surface only affects the initial few orders of Fourier coefficients, and the refinement program uses only higher-order Fourier coefficients and also corrects for the initial Fourier coefficients as well as the error in the estimation of the background. Under these circumstances, the background level was taken as that at which the intensity became uniform and this was subtracted from all points. The scattering angle was transformed to $\sin\theta/\lambda$ and the Fourier coefficients were calculated from these intensity data after they were corrected for Lorentz and polarization factors.

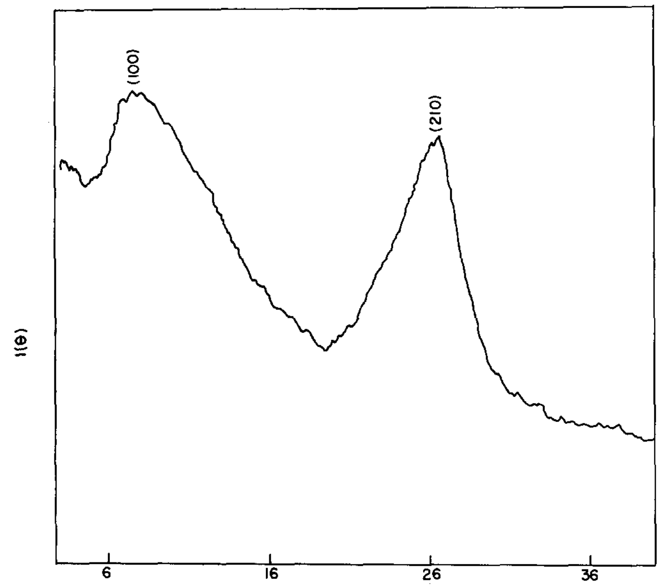

Figure 1 X-ray diffractometer recording of Nistari silk fibre ($I(\theta)$ in arbitrary units, 2θ in degrees)

In order to correct the instrumental line broadening using the Stokes method[12] the X-ray diffraction pattern was recorded for aluminium foil under the same conditions as used for silk fibres. The procedure was repeated for all the samples annealed at various temperatures for various time periods.

The calculation of the intensity profile using equations (1), (2), (3) and (5) and an exponential distribution function for crystal size requires four parameters, namely lattice disorder g, crystal size $\langle N \rangle$, error in background and a parameter α defining the width of the distribution function of column lengths. Initial values of g and $\langle N \rangle$ were obtained using the method of Nandi *et al.*[13]. Using these values in the above-mentioned equations gave the corresponding values for the distribution width. These are only rough estimates but the refinement procedure must be sufficiently robust to start with such inaccurate values.

Here we compute:

$$\Delta^2 = [I_{\text{calc}} - (I_{\text{exp}} + BG)]^2/(\text{number of points})$$

The value of Δ was divided by half the maximum value of intensity so that it is expressed relative to the mean value of intensities and this function is minimized. For refinement a multi-dimensional minimization algorithm of the Simplex method was used[14]. Here BG refers to the inaccuracy in the background estimation. It was observed that the variation of $\langle N \rangle$, p and α defined in equation (3) with respect to g for an exponential distribution is almost constant and, under these circumstances, the average values of parameters $\langle N \rangle$, p and α were used to determine the g value; these are given in *Table 1*. All the necessary computer programs were written in FTN77 language and were compiled and executed using an Archimedes, 310M, Acorn Computer.

Table 1 Paracrystalline parameters obtained from (1 0 0) and (2 1 0) reflections of pure Nistari silk fibres

		(1 0 0) reflection, $d_{100} = 13.86$ Å				(2 1 0) reflection, $d_{210} = 4.29$ Å			
No.	Sample	$D = \langle N \rangle d$	P	g (%)	A (0)	$D = \langle N \rangle d$	P	g (%)	A(0)
1	Pure Nistari	25.47	1.80	6.4	215.8	22.99	1.89	1.0	36.2
		±0.4	±0.1	±0.1		±1.0	±0.07	±0.1	
2	Annealed 100°C, 7 h	27.01	1.85	7.4	193.0	25.17	3.06	2.5	53.2
		±0.3	±0.04	±0.4		±0.9	±0.13	±0.2	
3	Annealed 100°C, 14 h	23.79	1.68	8.0	202.0	25.82	3.23	5.7	52.0
		±0.3	±0.02	±2.0		±1.0	±0.15	±1.0	
4	Annealed 140°C, 7 h	24.42	1.73	8.0	206.0	21.35	2.76	5.8	48.3
		±0.1	±0.05	±3.0		±0.7	±0.09	±0.2	
5	Annealed 140°C, 14 h	26.32	1.70	8.0	176.0	24.42	3.77	5.2	39.4
		±0.2	±0.10	±2.0		±0.9	±0.18	±0.3	
6	Annealed 200°C, 7 h	26.69	1.62	9.0	180.0	23.89	2.57	2.2	43.9
		±0.3	±0.05	±3.0		±1.0	±0.12	±0.3	

RESULTS AND DISCUSSION

In order to verify that we have estimated the actual crystal size, in *Figure 2* we have compared our results with Vainshtein's equation[15] $g = 0.4(1/\langle N \rangle)^{1/2}$. It can be seen from the figure that, if a correlation effect is present, then it should be characterized by points that lie along the straight line passing through the origin with a slope of 0.4. The very fact that the points are far away from the line indicates that there is no correlation effect.

Table 1 gives the parameters needed to recalculate the profile of (1 0 0) and (2 1 0) reflections using equations mentioned earlier in the text. *Figure 3* shows good agreement between the experiment and the intensity calculated on the basis of the paracrystalline model suggested in this paper for (1 0 0) and (2 1 0) reflections in Nistari silk fibres annealed at different temperatures. From *Table 1* we can conclude that the crystal size has maximum values along (1 0 0) and (2 1 0) directions when the fibre is annealed at 100°C for 7 h. The increase in crystal size with annealing temperature is due to a recrystallization process at higher temperatures, which results in the parallel alignment of the protein molecules (β-pleated structure).

The sharpening of the peaks can also be expected because the crystallites are now strain-free and sometimes the distortion reduced with annealing at higher temperatures. For temperatures above 150°C there is a marked transition internally, which is due to the reorientation of Ser–Ala–Gly labile groups within the crystal region. But the variation in crystal size value is small because of the increase in the amorphous region. At 200°C annealing temperature, we have also noticed external changes like slight decoloration, but without any significant changes in the crystal size value.

It may be recalled that the (1 0 0) reflection arises from the planes whose normals are in the direction of the side chains of the amino acid residues. Along the (1 0 0) direction the distortion and crystal size values increase with increase in annealing temperature, which is due to the partial realignment of the protein molecules. This is true even along the (2 1 0) direction. It is puzzling to note that there is an increase in lattice distortion along (1 0 0) and (2 1 0) directions with increase in annealing temperature and this may be due to the limitations of the exponential distribution model used here to separate out the size and distortion coefficients from the Fourier coefficients. It is useful to note that the crystal size along (2 1 0) directions is greater for Nistari compared with pure Mysore silk[10] even though Nistari and pure Mysore silk come from multivoltine races. This difference has not so far been reported.

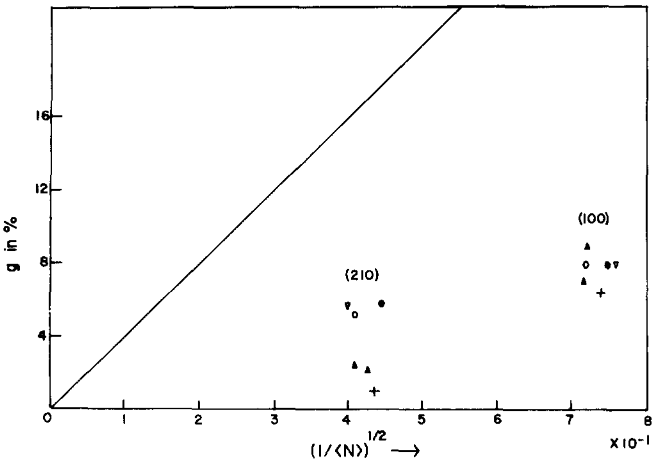

Figure 2 Variation of g with $(1/\langle N \rangle)^{1/2}$ for Nistari silk fibre: samples 1 (+), 2 (▲), 3 (▽), 4 (●), 5 (○) and 6 (△) as in *Table 1*

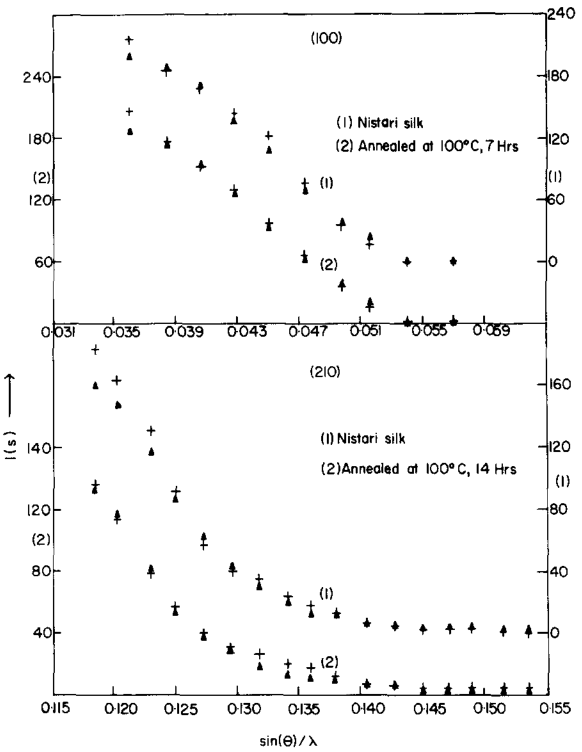

Figure 3 Calculated (▲) and observed (+) intensities for (1 0 0) and (2 1 0) reflections

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