

# Elastic modulus of the crystalline regions of silk fibroin

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The elastic modulus  $E_1$  of crystalline regions of silk fibroin of *Bombyx mori* (silk II) parallel to the chain axis was determined by X-ray diffraction. The  $E_1$  values for both the (002) and (006) plane ( $c$  axis; chain direction) of silk II coincided with each other and were obtained as 23 GPa. This small  $E_1$  value was due to the skeletal conformation of silk II being contracted 5.6% compared with the fully extended planar zig-zag conformation, and the deformation of the chain was mainly performed through the change of internal rotation angle with a small force constant. The specimen modulus was 10 GPa. This value was 43.5% of the  $E_1$  value and the elasticity of elongation work was almost unity after undergoing the recycle test five times. This means the macroscopic deformation of the specimen was largely influenced by the crystal elasticity.

(Keywords: elastic modulus; crystalline regions; silk fibroin; crystal modulus; mechanical properties)

## INTRODUCTION

The elastic moduli of polymer crystals give us important information on molecular conformation in the crystal lattice<sup>1-6</sup>. We have been engaged in measuring the elastic moduli  $E_1$  of various polymer crystals by X-ray diffraction parallel to the chain axis. Examinations of the data so far accumulated led us to success in relating the  $E_1$  value, namely the extensivity of a polymer molecule, both to the molecular conformation and the mechanism of deformation in the crystal lattice. The values of  $E_1$  for polymers with a fully extended planar zig-zag conformation, i.e. polyethylene (PE) and poly(vinyl alcohol) (PVA) are found to be high (235 and 250 GPa, respectively). The extension of a molecule in these cases occurs by bond stretching and bond angle bending. While  $E_1$  values for polymers with contracted conformation in the crystal lattice are smaller, i.e. 35 and 2.59 GPa for isotactic polypropylene and poly(trimethylene terephthalate)<sup>7</sup>, respectively. In these cases, the extension of a molecule involves internal rotation around the single bond with a small force constant.

Further, knowledge of the elastic modulus  $E_1$  of polymers is of interest in connection with mechanical properties of polymers, because  $E_1$  gives us the maximum value for the specimen modulus of a polymer.

Silk fibre is one of the most important fibrous polypeptides, and nowadays it is very useful not only for textiles, but also for binding enzymes etc.<sup>8</sup>. Since the appearance of nylons, many other synthetic fibres including silk-like fibre have come into use. However, silk textile is still valued because of its unparalleled qualities, such as glossiness, liveliness, good recovery behaviour from deformation, and agreeableness to the touch etc.<sup>9</sup>. These qualities are connected with the mechanical properties of silk fibroin, and so it is interesting to investigate them with respect to  $E_1$ .

The crystalline regions of silk fibroin of *Bombyx mori* consist of glycine, alanine and serine residues (3:2:1)<sup>10</sup>. It has been reported that the molecular chain of silk fibroin has a large scale zig-zag conformation in which the zig-zag unit is composed of amino acid residues, and is shrunk 5.6% compared with the fully extended planar zig-zag conformation. These adjacent chains are linked by hydrogen bonds to form the so-called antiparallel chain plated sheets structure<sup>11</sup>. This is one of the most typical structures for proteins. Therefore, the other purpose in measuring the  $E_1$  value of silk fibroin is to obtain information on the mechanical properties of the  $\beta$ -sheet structure.

In this paper, we measure the elastic modulus  $E_1$  of silk fibroin by X-ray diffraction, and discuss its relationship with crystal structure and specimen modulus.

## EXPERIMENTAL

Degummed and annealed silk fibroin of *Bombyx mori* was used. To remove sericin which cemented the fibroin filaments together, the raw fibre was washed at 70–80°C for 30 min with 0.5% *Marseilles Soap* and 0.5%  $\text{Na}_2\text{CO}_3$  aqueous solution, then rinsed with deionized water and air dried. The degummed silk fibroin was annealed at 200°C for 60 min at constant length. The density of the degummed and annealed specimen was  $1.375 \text{ g cm}^{-3}$  measured by the flotation method in benzene-carbon tetrachloride at 30°C.

Figure 1 shows the meridional diffraction profile of silk fibroin obtained using an X-ray diffractometer (Rigaku Denki Co. Ltd, RAD-B System). Three reflections were observed at  $2\theta = 25.4^\circ$ ,  $39.7^\circ$  and  $83.2^\circ$ , respectively. Marsh *et al.*<sup>11</sup> reported that the crystal structure of silk fibroin of *Bombyx mori* (silk II) belongs to a monoclinic system and that the unit cell parameters are  $a = 9.44$ ,

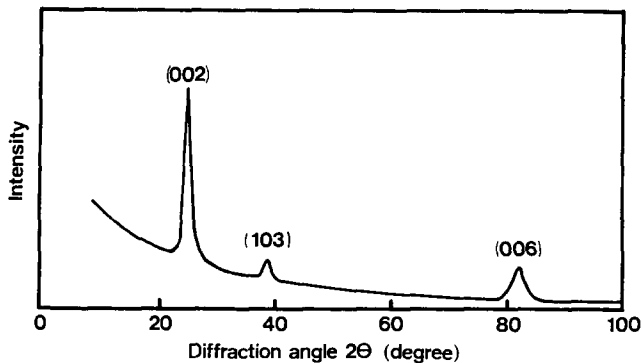


Figure 1 Meridional diffraction pattern of silk fibroin

$b = 9.20$  and  $c = 6.97 \pm 0.03$  Å (fibre axis is converted from  $b$  to  $c$  axis) and  $\beta = 90^\circ$ . Using these data, the X-ray peaks located at  $2\theta = 25.4^\circ$  and  $83.2^\circ$  can be indexed as meridional reflections (002), (006), respectively. The peak at  $2\theta = 39.7^\circ$  is an off-meridional reflection indexed as (103) calculated from the unit cell parameters and its inclination angle ( $13.9^\circ$ ) from the fibre axis. There are a few reports<sup>11</sup> that reflections from the serine residue appear at a lower angle of  $2\theta$  but such reflections were not observed in this study.

Thus, these two meridional reflections of silk II were used for the measurement of  $E_1$ .

The lattice extension under a constant load was measured by means of an X-ray diffractometer equipped with stretching device. The strain  $\varepsilon$  in the crystalline regions was estimated by use of the relation

$$\varepsilon = \Delta d / d_0$$

where  $d_0$  denotes the initial lattice spacing, and  $\Delta d$  is the change in lattice spacing induced by a constant stress. The experimental error in measuring the peak shift was evaluated ordinarily to be less than  $\pm 1/100^\circ$  in the angle  $2\theta$ .

The stress  $\sigma$  in the crystalline regions was assumed to be equal to the stress applied to the sample. This assumption of a homogeneous stress distribution has been proven experimentally for PE, PVA and cellulose<sup>1-6</sup>.

The elastic modulus  $E_1$  was calculated as

$$E_1 = \sigma / \varepsilon$$

A more detailed description of the measurements has been given in earlier papers<sup>1-6</sup>.

The determination of crystallite size and lattice distortion of silk II was performed by measuring meridional profiles of the (002) and (006) reflections. Observed integral widths  $B_0$  of these diffractions are defined as follows:

$$B_0 = \int I(2\theta) d(2\theta) / I_{\max}$$

where  $2\theta$  is the reflection angle on which the reflection is portrayed. The correction for the doublet,  $\text{CuK}_{\alpha 1}$  and  $\text{K}_{\alpha 2}$ , broadening was carried out by the Jones' method<sup>12</sup> and the corrected values are designated as  $B$ . Further, the values of  $B$  were corrected for instrumental broadening  $b$  according to the equation

$$\beta^2 = B^2 - b^2$$

where  $\beta$  is the pure integral width of the reflection. The integral width ( $\delta S$ ) on reciprocal-lattice space was given as follows:

$$\delta S = \beta \cos \theta / \lambda$$

where  $\theta$  is the Bragg angle and  $\lambda$  is the wavelength of the X-ray.

Silk II shows only two meridional reflections, (002) and (006). The lattice distortion was assumed to be paracrystalline type and  $\delta S$  can be expressed by the so-called Hosemann's plot<sup>13</sup>.

$$(\delta S)^2 = (1/D^2) + (\pi^4 g_{\parallel}^4 m^4 / d^2)$$

where  $m$  is the order of reflection and  $d$  is the fibre identity period. The crystallite size  $D$  and the paracrystalline distortion  $g_{\parallel}$  in the direction parallel to the chain axis were obtained from the intercept and slope of the  $(\delta S)^2 - m^4$  plots<sup>14</sup>.

The stress-strain curve for the specimen was measured by a tensile tester (Shimadzu Autograph SD-100). The initial length of the specimen was 30 mm and the extension rate was  $3 \text{ mm min}^{-1}$ .

All measurements were performed at  $25^\circ\text{C}$  (60% relative humidity).

## RESULTS AND DISCUSSION

Figure 2 shows the diffraction intensity for the (002) reflection of silk II as a function of the inclination angle of the incident X-ray beam to the fibre axis. The degree of orientation  $\pi$  defined by

$$\pi = (180 - H^\circ) / 180$$

is 0.94 both at  $\sigma = 0$  and 100 MPa, where  $H^\circ$  is the half-width of the intensity distribution curve along the Debye-Scherrer ring of the (002) reflection (Figure 2). Further, the diffraction intensity did not change owing to the stress. This means that the degree of orientation is constant and the crystallites are not broken by stress up to 100 MPa.

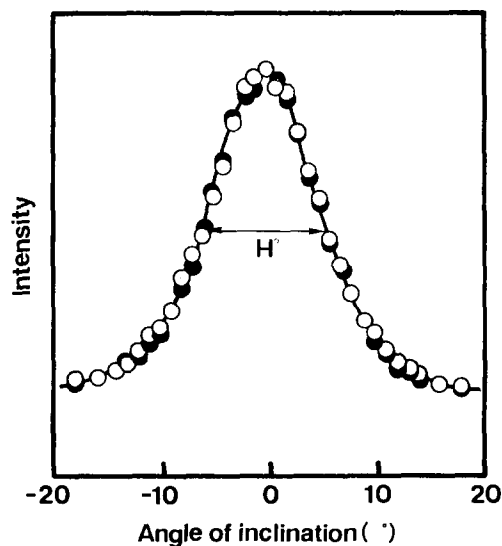


Figure 2 Diffraction intensity vs. the angle of inclination for the (002) plane of silk fibroin. At 0 (○) and 100 MPa (●)

Figure 3 shows the stress ( $\sigma$ )–strain ( $\epsilon$ ) curve for the (002) plane of silk II. The lattice extension is linear up to 200 MPa and the slope gives a value for  $E_1$  of 23 GPa. The  $f$ -value, the force required to stretch a molecule by 1%, calculated from both  $E_1$  and the cross-sectional area ( $21.7 \text{ \AA}^2$ )<sup>11</sup> of one molecule in a crystal lattice, is  $0.50 \times 10^{-5}$  dyne for silk II. The observed  $E_1$  value (23 GPa) for silk II is about one-tenth of those for PE (235 GPa) and PVA (250 GPa)<sup>1–6</sup>, which have planar zig-zag conformation in the crystalline regions. The  $f$ -value for silk II is also smaller than those for PE or PVA.

The molecular structure of silk II, proposed by Marsh *et al.*<sup>11</sup>, is shown in Figure 4. The molecular chain has a large scale zig-zag conformation, in which the zig-zag unit is composed of amino acid residue, and is shrunk 5.6% compared with the fully extended planar zig-zag conformation owing to the internal rotation angles ( $\phi_1$ ,  $\phi_2$ ,  $\phi_3$ ). These internal rotation angles ( $\phi_1$ ,  $\phi_2$ ,  $\phi_3 = -142^\circ$ ,  $180^\circ$ ,  $145^\circ$ ) of silk II resemble those of

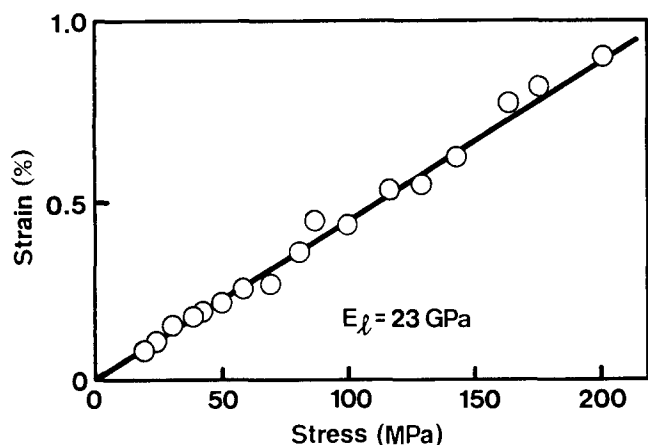


Figure 3 Stress–strain curve for the (002) plane of silk fibroin

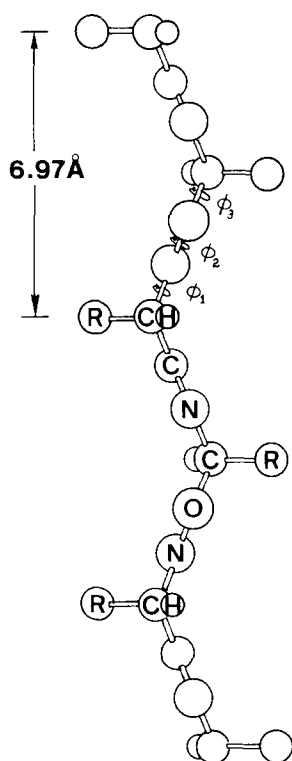


Figure 4 Molecular structure of silk fibroin II after Marsh *et al.*<sup>11</sup>

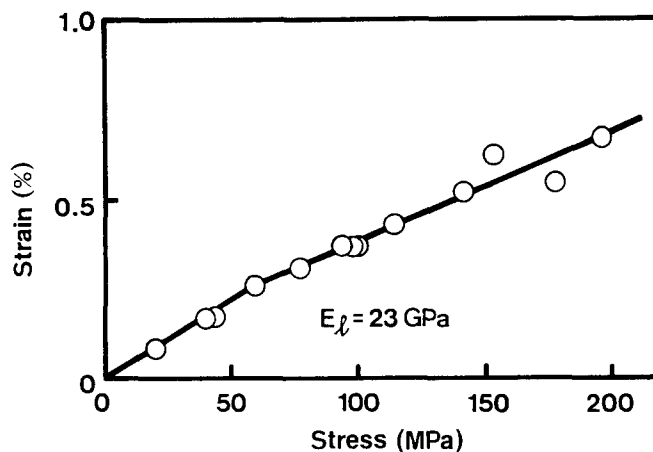


Figure 5 Stress–strain curve for the (006) plane of silk fibroin

poly(isobutylene oxide) (PIBO), that is  $\phi_1$ ,  $\phi_2$ ,  $\phi_3 = -153^\circ$ ,  $180^\circ$ ,  $153^\circ$  (ref. 14). It has been reported that the  $E_1$  value depends only on the skeletal conformation, that is, the deformation mechanisms of a molecule, and the species and sizes of side chains scarcely affect the  $E_1$  value whether strong interaction exists between neighbouring chains or not. So, it is reasonable that the  $E_1$  value (23 GPa) of silk II is close to that for PIBO ( $E_1 = 29.3$  GPa)<sup>15</sup> despite their chemical structures differing greatly. The  $E_1$  value of silk II was calculated to be 160 GPa<sup>16</sup> using Treloar's method<sup>17</sup>. This value would be too large compared with the observed  $E_1$  value, because Treloar's method does not contain the effect of the change of internal rotation angle. Thus, the small  $E_1$  and  $f$ -value of silk II can be attributed to the fact that the chain has a contracted structure and the mechanism of chain deformation is mainly due to the change of rotation angle, with the force constant being smaller than that of bond stretching and angle bending. For the polymer, an inherent  $E_1$  value should be obtained independent of the order of reflections. However, for Nylon 6  $\alpha$ -form, different orders of meridional reflections give different  $E_1$  values<sup>18</sup>. When the crystallite size along the chain direction is small and changes with the applied stress, the diffraction peak shifts not only by lattice extension, but also by change in the Laue lattice factor. The different  $E_1$  values for Nylon 6 were found to be due to the increase in the crystallite size with the applied stress. By correcting for the Laue lattice factor, the values of  $E_1$  measured for other reflections agreed well with each other. Next we checked the lattice extension of silk II by using the (006) plane.

Figure 5 shows the stress ( $\sigma$ )–strain ( $\epsilon$ ) curve for the (006) plane of silk II. The lattice extension  $\epsilon$  seems to level off at higher stress (above 100 MPa) despite the lattice extensions always being reversible. However, from the initial slope of the curve,  $E_1$  is 23 GPa for the (006) plane which is in accordance with the  $E_1$  value for the (002) plane.

Table 1 shows the crystallite size  $D$  and the lattice distortion  $g_{11}$  of silk II before, under stress ( $\sigma = 200$  MPa) and after unloading.  $D$  and  $g_{11}$  before loading are 106 Å and 1.7%, respectively. However,  $D$  decreased and  $g_{11}$  increased with stress (200 MPa), each value is recovered after unloading. It may be considered that the crystallite size is sufficiently large that the extent of change in crystallite size does not cause the peak shift owing to the

**Table 1** Effect of applied tensile stress on the crystallite size and lattice distortion in the direction parallel to the chain axis of silk fibroin

Stress (MPa)	Crystallite size (Å)	Lattice distortion (%)
Before loading	106	1.7
200	89	1.9
After unloading	108	1.7

change of Laue lattice factor for silk fibroin. Consequently, the phenomenon which occurred for Nylon 6 was not observed for silk II. We propose that the inherent elastic modulus  $E_1$  of crystalline regions parallel to the chain axis of silk II is 23 GPa.

The tensile strength and the specimen modulus  $Y_1$  of the specimen of silk fibroin, measured by tensile tester, are 330 MPa and 10 GPa, respectively. The proportion of  $Y_1$  to  $E_1$  ( $Y_1/E_1$ ) is 43.5%. This percentage is large in comparison with common synthetic fibres where the value of  $Y_1/E_1$  is usually up to 30%, except for rigid-rod polymers such as Kevlar etc.<sup>6</sup>. Thus, a large  $Y_1/E_1$  value indicates that the molecular chains of silk fibroin in the amorphous regions are somewhat in the oriented state and also linked by hydrogen bonds. No peak using small angle X-ray scattering also indicates the ordered state of amorphous regions.

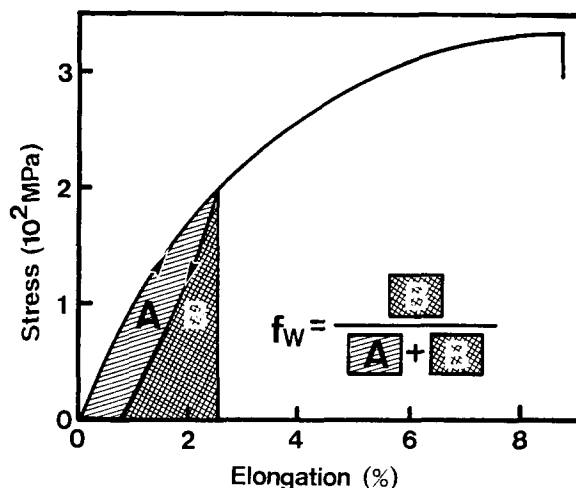
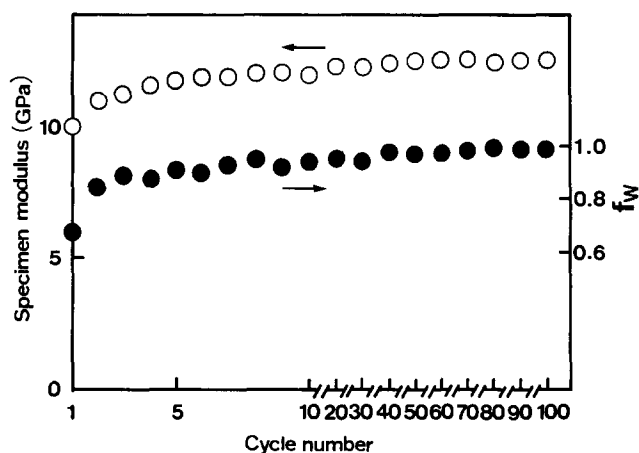
Iizuka reported that the dynamic storage modulus (170 Hz) and crystallinity increased with decreasing the size of silk thread<sup>16</sup>. When extrapolating the size of silk thread to zero, a  $Y_1$  value of 187 g dyn<sup>-1</sup> was obtained. Assuming that the density is 1.37 g cm<sup>-3</sup>, this value can be converted to 22.6 GPa, which is very close to the  $E_1$  value (23 GPa).

The area under the stress-strain curve corresponds to the energy required to stretch the silk fibroin (Figure 6). The parameter of elastic recovery is now defined as the elasticity of elongation work<sup>19</sup>

$$f_w = S_B / (S_A + S_B)$$

the proportion of area between at tensile stress ( $S_A + S_B$ ) and at recovery ( $S_B$ ) on the stress-strain curve is schematically shown in Figure 6. The recycle test is performed, for example, with the fixed conditions that the tensile stress changed between 0 MPa and 200 MPa, 60% of the breaking stress.

Figure 7 shows the relationships between the recycle number and the specimen modulus  $Y_1$  and the parameter  $f_w$  of elastic recovery of silk fibroin. The initial specimen modulus of silk fibroin is 10 GPa, and this value increases with recycle number. After repeating five times,  $Y_1$  became constant at 12 GPa. It has been shown that the degree of the crystallite orientation does not change, even if silk fibroin is stretched as much as possible<sup>16</sup>. The increment of specimen modulus will be due to the chain extension in amorphous regions by the drawing effect of the recycle test. Further,  $f_w$  is nearly unity after repeating the test five times. This shows that the elastic recovery of silk fibroin is almost complete. This means that the mechanical properties of silk fibroin are strongly controlled by the elasticity of crystallites, and this gives the special mechanical properties of good flexibility and recovery.

**Figure 6** Stress-elongation curve for the specimen of silk fibroin and schematic recovery curve.  $f_w$  denotes the elasticity of elongation work**Figure 7** Relationship between recycle test number and (O) specimen modulus, (●)  $f_w$  of silk fibroin

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