

Structural Changes and Dyeability of Silk Fibroin Fiber Following Shrinkage in Neutral Salt Solution

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SYNOPSIS

The fine structural changes of *Bombyx mori* silk fibroin fibers induced by shrinking with concentrated calcium chloride aqueous solution at elevated temperature were investigated as a function of shrinking rate. Tensile strength decreased and elongation at break increased in the shrinkage range 13–67%, the shape of the stress–strain curve changing from rubber-like to brittle at high shrinkage values (70–90%). The birefringence gradually decreased over the entire shrinking range examined, the curve becoming steep as the shrinkage exceeded about 67%. The behavior of isotropic refractive index ($n_{i_{iso}}$) closely resembled that of birefringence (Δn) in the shrinking range 13–67%. Beyond shrinkage of 67%, the $n_{i_{iso}}$ showed a tendency to increase, especially for the sample with 80% shrinkage. Dichroism measurements showed that the molecular orientation within the amorphous regions decreased sharply at the beginning of the shrinking treatment, within the range 0–13%, then attained a saturation at about 55%. The position and intensity of the major X-ray diffraction peak at 20.5° remained essentially unchanged regardless of the shrinking treatment. The results of dyeing behavior showed that the saturation value attained by shrunk silk fibers was significantly larger than that of the untreated control sample. Both standard affinity and the heat of dyeing increased slightly for the shrunk silk fibroin fiber, suggesting that a larger number of reactive sites became available for the interaction between dye molecules and fibroin chains. A schematic model is proposed for explaining the relation between structural changes and enhanced dyeability of the silk fibers following shrinkage in neutral salt solution. © 1994 John Wiley & Sons, Inc.

INTRODUCTION

Degummed silk fibers, treated with concentrated aqueous solutions of neutral salts, tend to swell and shrink significantly, the extent of shrinkage being closely related to the treatment conditions, such as temperature, time, type, and concentration of salt, etc. This effect is mainly due to the weakening of the intermolecular hydrogen bonds between adjacent fibroin chains in the less ordered regions of the fibers. The ultimate effect of the above treatment is the complete dissolution of the silk fiber, which is

attained quite easily at room temperature with saturated solutions of some lithium (Li) salts.¹ In order to elucidate the mechanism of silk fiber shrinking by hydrogen bond reagents, Murase and Sakaguchi² found that the extent of shrinkage decreased considerably when the tyrosine residues of silk fibroin were chemically modified by reaction with cross-linking agents, such as aldehydes.

Leveau³ studied the shrinking behavior of silk fibers treated with lithium bromide (LiBr) solutions, and elucidated the effect of salt concentration and temperature, as well as the influence of several chemical pretreatments on the kinetics of fiber shrinkage and on the reversibility of the process. The mechanism of silk fibroin dissolution by action of calcium chloride (CaCl₂) aqueous solutions has

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recently been investigated by means of high resolution NMR techniques. Asakura and Nakayama⁴ observed ¹³C NMR spectra of *Bombyx mori* silk fibroin in a CaCl₂ aqueous solution as a function of the CaCl₂ concentration and detected that the NMR spectra changed markedly beyond 3.5 M of CaCl₂ concentration.⁴

There exists a considerable interest in exploiting the shrinkage treatment for the production of new silk goods with special properties, such as higher comfort and feel. Crepe-like silk fabrics have already been obtained by treating silk plane fabrics (Habutae) with concentrated salt solutions at high temperature. Kato⁵ recently reported the optimum conditions for preparing shrunk silk fibers to be used for knitted ladies summer dresses, whose mechanical properties related to the feel and comfort evaluation were investigated by KES measurements. Another practical interest in swelling agents, as well as in silk fiber shrinking, lies in the fact that this treatment can improve the dyeability of silk fibers by selective modification of their fine structure.

The aim of this article is to investigate the fine structural changes of silk fibers induced by treatment with concentrated CaCl₂ aqueous solution at high temperature. The degree of crystallinity and molecular orientation have been evaluated by measuring the tensile and optical properties (refractive indices, and dichroism), as well as the x-ray diffraction intensity curves as a function of the shrinking rate. The dyeing behavior was examined by determining various parameters, such as saturation absorption, standard affinity, and heat of dyeing of shrunk silk fibers dyed with two acid dyes at different temperatures.

EXPERIMENTAL

Materials

Bombyx mori silk fiber was subjected to swelling and shrinking in concentrated calcium chloride (CaCl₂) aqueous solution (9 M) at 98°C for different treatment times until certain shrinkage was attained. The silk fiber with different extents of shrinkage (0, 13, 55, 67, 80, 90%) was thus prepared. The shrinking rate of silk fiber was measured on the basis of the changes in the fiber length following shrinkage in CaCl₂ solution.

Measurements

The tensile properties of the shrunk silk fibers were measured with a Tensilon UTM-II (Toyo Baldwin

Co.) in standard conditions (20°C and 65% relative humidity, RH) at a gauge length of 50 mm and strain rate of 20 mm/min. Each value is the average of 25 results.

The refractive indices of shrunk silk fibers were measured with the Beche's line method using a polarized microscope under the monochromatic light (Na light) at 20°C and 65% RH as previously described.^{6,7}

The value of dichroism (f_D) was obtained for silk fibers according to eq. (1) using an Olympus microscopic photometer equipped with polarizer and analyzer. Silk fibers were first dyed at 70°C for 1 h with aqueous solution (1000 mL), containing 0.1 g Acid Blue 113, 3 mL acetic acid solution, and 10 g Na₂SO₄ 10 H₂O. Dichroism value (f_D) was estimated by measuring the intensity of the polarized light parallel (K) and perpendicular (K') to the fiber axis.

$$f_D = (K - K') / (K + K'). \quad (1)$$

Dichroism is a convenient measure to evaluate the extent of the silk fibroin molecular orientation in the amorphous region but not in the crystalline region. Dichroism measurements are based on the evaluation of the orientation of the dye molecules attached and inserted within the amorphous region.

Optical orientation f was evaluated according to the equation

$$f = \Delta n / I_u \times 100 (\%). \quad (2)$$

in which Δn and I_u represent the birefringence of the silk fiber with different extents of shrinkage and the ideal birefringence value (0.068) corresponding to the perfectly oriented fibroin molecules.

X-ray diffraction patterns were obtained using an x-ray source with CuK α radiation ($\lambda = 1.54 \text{ \AA}$). The conditions for the x-ray measurements have been described in detail elsewhere.⁷ The degree of crystallinity of the shrunken silk fiber was evaluated on the basis of the x-ray diffraction intensity curves according to the method described by Hermans and Weidinger.⁸

The dyeability behavior of silk fibers was examined as follows: Saturated adsorption, standard affinity, and heat of dyeing in the dyeing process of the shrunk silk fiber were calculated by employing the equations proposed by Gilbert-Rideal⁹ and Kato.¹⁰ These parameters were obtained by measuring the amount (D)₀ of adsorption of dye anion, and the concentration (H)₀, (D)₀ of hydrogen ion and dye anion in the dyeing bath, respectively. Samples were dyed using a dye-o-meter (STD-3P

type) of Suga Test Instrument Co., Ltd. About 1 g of silk fiber with 15% shrinkage was sealed in the Pyrex tube containing the dye bath with 2% dye A. Orange 7 (Orange II, Tokyo Kasei Co.) or A. Red 18 (Kayaku Acid Brilliant Scarlet 3R, Nihon Kasei, Co.) on the basis of the fiber weight, keeping a material-to-liquor ratio of 1:300. The dyeing was started at 25°C. The dyeing temperature was gradually increased from 40 to 90°C over 90 min and was maintained at the same temperature for 135 min.

RESULTS AND DISCUSSION

Tensile Properties

Because tensile properties are important characteristics that determine the behavior of textiles during industrial processing as well as the functional performances of the final product, we studied the stress-strain curves of shrunk silk fibers in relation to the shrinking rate (Fig. 1). The stress of the specimen was normalized for denier, which is a measure of cross sectional area. The untreated control fibers [Fig. 1(a)] are characterized by fairly high strength and breaking extension, which combine to give an appreciably high work of rupture. The shape of the curve [Fig. 1(a)] is typical of a fibrous material with a rather high degree of molecular orientation. As the elongation at break increased, the breaking

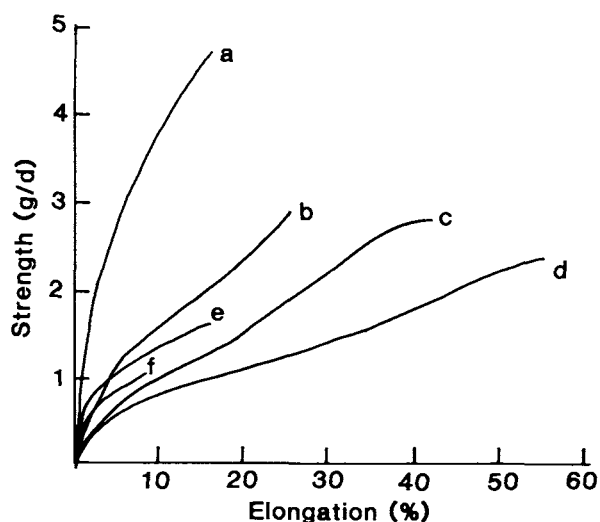


Figure 1 Tensile strength and elongation at break of the silk fibers with different extents of shrinkage. Shrunk silk fibers were prepared by immersing in very concentrated CaCl_2 solution (9M) at 95–98°C for different reaction time. Shrinking rate (%): (a) 0; (b) 13; (c) 55; (d) 67; (e) 80; (f) 90.

strength shifted to lower values for the treated silk fibers in the shrinking rate range 13–67%. As the amount of shrinking rate increased from 13 to 67% [Fig. 1(b–d), respectively], the stress-strain curve changed significantly, resembling that of fibrous material with poorly oriented structure. The slopes estimated from the stress-strain curves for shrunk samples with higher shrinking rate [Fig. 1(e, f)], corresponding to the tensile modulus, became steep compared with those of the shrunk samples with lower shrinking rate when the extent of shrinking rate increased. In addition, a yield point at low strength appeared, above which the fibers elongated quite easily.

The mechanical behavior of shrunk silk fibers closely resembles that of tussah silk,¹¹ whose tensile properties have been attributed to the lower degree of molecular orientation both in the amorphous and laterally ordered regions. This suggests that the primary effect of the shrinking treatment is the disordering of the fibroin molecules in the amorphous regions. This effect is supposed to extend gradually to the well-oriented laterally ordered regions,⁷ as the extent of shrinkage exceeds a certain limit. Silk fibers with 80% and 90% shrinkage behaved quite differently compared with the other shrunk samples [Fig. 1(e, f)]. The stress-strain curves [Fig. 1(e, f)] changed from rubber-like to brittle. The slopes of the shrunk specimen [Fig. 1(e, f)] are steeper than those of the less shrunk samples [Fig. 1(b–d)], but their strengths and elongations at break are very low. The drastic drop of the mechanical properties should be attributed both to the degradative effect due to the solvent system used to attain the ultimate degree of shrinking, and to an extensive modification of the arrangement of the fibroin chain. This glassy and tough fibrous structure can be represented as a closely packed three dimensional network of fibroin polypeptides, stabilized by inter- and intramolecular hydrogen bonds. Extensive modifications of both amorphous and crystalline regions should be considered as well.

Refractive Indices

The average degree of orientation and crystallinity of silk fibers treated with CaCl_2 aqueous solution were evaluated by measuring the refractive indices. Figure 2 shows the birefringence (Δn) and isotropic refractive index (n_{iso}) as a function of the shrinking rate. The Δn value decreased over the entire shrinking range examined, the curve becoming steep until the shrinkage exceeded about 67%. A sharp drop of the curve was also noted at a very early stage of shrinking (0–5%) in response to the initial treat-

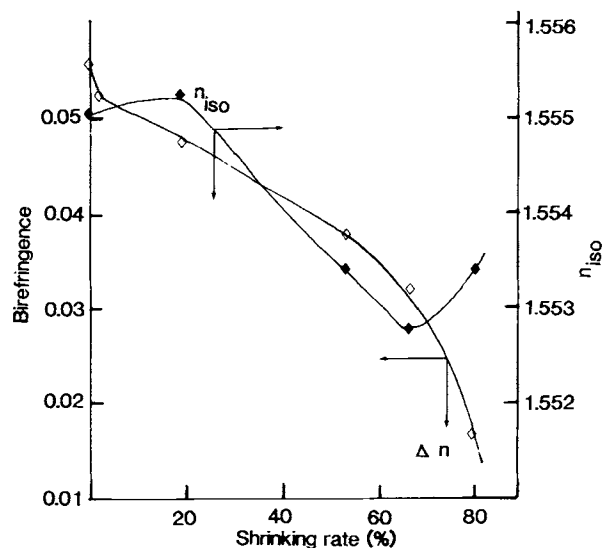


Figure 2 Birefringence (Δn) and isotropic refractive index (n_{izo}) of the shrunk silk fibers as a function of the shrinkage.

ment with the salt solution. These data agree with the mechanical behavior [Fig. 1(b-d)]. The larger the shrinkage, the lower the molecular orientation, and the more the stress-strain curve differs from that of the untreated control sample, in such a way that shrunk silk fibers behave as a rubber-like polymer.

The behavior of the isotropic refractive index closely resembles that of birefringence in the shrinking range 13–65%. Beyond the shrinkage of 67%, the n_{izo} values showed a tendency to increase, especially for the sample with 80% shrinkage. Although the initial rise of n_{izo} is comparatively slight and can be interpreted as an increase of fiber density due to the contraction of the fibroin molecules in the amorphous regions, the increase in n_{izo} registered at 80% shrinkage should imply more drastic changes in the fine structure of shrunk silk fibers, and should be related to the brittleness exhibited by the corresponding sample during the mechanical tests [Fig. 1(e)].

Dichroism

The fine structural changes of silk fibers subjected to shrinkage were further investigated by measuring dichroism in the visible spectral region of the fibers dyed with Acid Blue 113. The significance of the dichroic ratio is that it may be quantitatively related to certain orientation functions characteristic of the average orientation of structural units within the

fibrous polymer. Because the dye molecules are exclusively located in the amorphous phase, dichroism measurements are capable of characterizing the average chain orientation within these regions. Figure 3 shows the behavior of the dichroic ratio as a function of the shrinking rate. It is interesting to note that the molecular orientation of the fibroin chains in the amorphous regions decreased sharply at the beginning of the shrinking treatment, within the range 0–20%, then showed a tendency to saturate beyond 40% of shrinking rate, above which value no significant changes were detected. However, the results of birefringence (Fig. 2) show that the decrease of molecular orientation is continuous over the entire shrinking range examined. We should therefore assume that other structural domains of silk are involved in fiber shrinking, and that their role is dependent on the extent of shrinkage attained. In order to further elucidate this behavior, we calculated the parameter f , that is, the ratio between the Δn value of shrunk silk fibers and that of the highly oriented crystalline and laterally ordered regions, which evaluate the contribution of the latter domains to the Δn changes as a function of the increase of shrinkage (Fig. 3). The curve shows that the decrease of f becomes larger at above 40–50% shrinkage, when the disordering within the amorphous regions has already occurred at its maximum extent. These findings imply that the shrinkage of silk fibers may be regarded as a sequence of phases, to which amorphous, laterally ordered,⁷ and crystalline regions contribute at a different extent. At the beginning, shrinkage is mainly governed by the structural

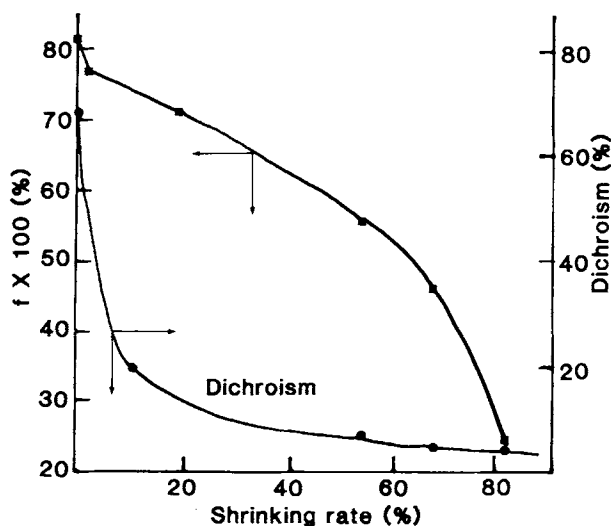


Figure 3 Optical orientation f and dichroism of the shrunk silk fibers as a function of the shrinking rate.

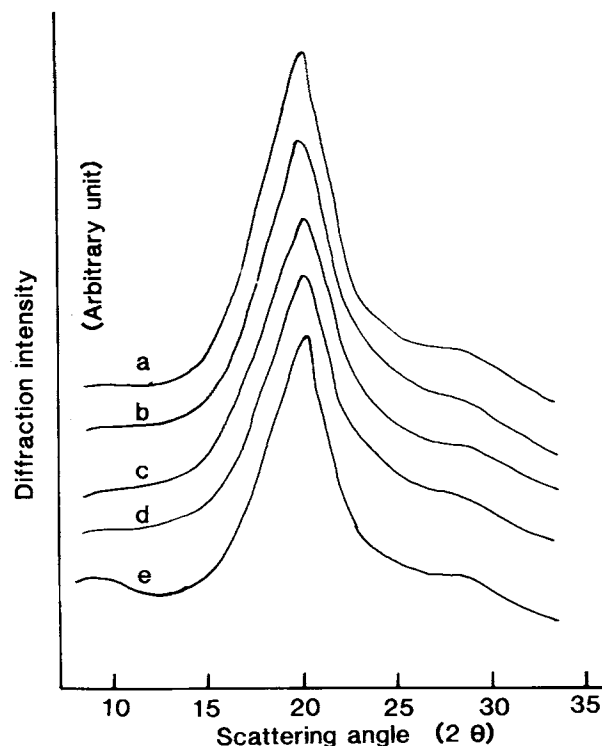


Figure 4 X-ray diffraction intensity curves of the silk fibers with different extents of shrinkage.

changes occurring in the amorphous regions, where the fibroin molecules are sufficiently free to move and rearrange in response to the immersion in concentrated CaCl_2 aqueous solution. As the extent of shrinkage increases, other fiber domains, that is, the laterally ordered region⁷ and probably the crystalline regions (see n_{iso} data), should play a major role.

X-Ray Diffraction Curves

The changes induced by shrinking in the crystalline regions of silk fibers were selectively studied by

measuring the x-ray diffraction curves (Fig. 4). The position and intensity of the major x-ray diffraction peak at 20.5° , corresponding to the specific crystalline spacing of 4.39 \AA characteristic of silk fibers with highly oriented β structure, remained essentially unchanged regardless of the shrinking treatment, even at 80% shrinkage [Fig. 1(e)]. The latter sample showed a minor peak appearing at small scattering angle, at about 7° , corresponding to silk I crystal structure. The degree of crystallinity of untreated and shrunk silk fibers, calculated from the x-ray diffraction intensity curves, was 24 and 22%, respectively. These results imply that the crystalline regions of silk fibers exhibit a certain inertness toward the shrinking treatment under the experimental conditions adopted; the intermolecular hydrogen bonds continue linking the crystalline polypeptide sequences of fibroin. The n_{iso} decreasing in the range 20–65% should be therefore attributed to the modification of crystal orientation and size, and/or to an indirect effect driven by the changes occurring within the laterally ordered regions.

Dyeing Behavior

It is generally assumed that the dyeing behavior of textile fibers is mainly governed by the accessibility of the amorphous regions, where the dye molecules diffuse and interact with the polymer chains by means of hydrogen bonds, electrostatic force, Van der Waals' force, and hydrophobic interactions. The results reported in this article imply that the shrinkage of silk fiber, induced by immersion in concentrated CaCl_2 solution, is effective in reducing the degree of order and orientation of the fiber. This fact may therefore suggest that new fiber domains become accessible to dye molecules, for example by selective disordering of the laterally ordered regions. From this point of view, the shrinkage treatment could become an interesting technological approach

Table I Saturated Adsorption, Standard Affinity, and Heat of Dyeing

Dyes	Sample	$(S)_\phi$			$-\Delta\mu$			ΔH^0
		40°C	60°C	80°C	40°C	60°C	80°C	
A. Orange 7	Treated	23.1	24.6	26.3	10.99	11.32	11.57	-7.2
	Control	20.0	20.4	21.1	10.89	11.11	11.43	-6.6
A. Red 18	Treated	21.8	22.4	24.6	22.63	23.88	24.70	-6.8
	Control	18.2	19.3	22.1	22.43	23.62	24.52	-6.2

Unit: $(S)_\phi$ ($\times 10^{-5}$ eq/g); $\Delta\mu$ (Kcal/mol); ΔH^0 (Kcal/mol). Shrunk silk fiber with 13% shrinkage, evaluated from measuring the amount of adsorption of dye anion, the concentration of hydrogen ion, and dye anion in the dyeing bath.

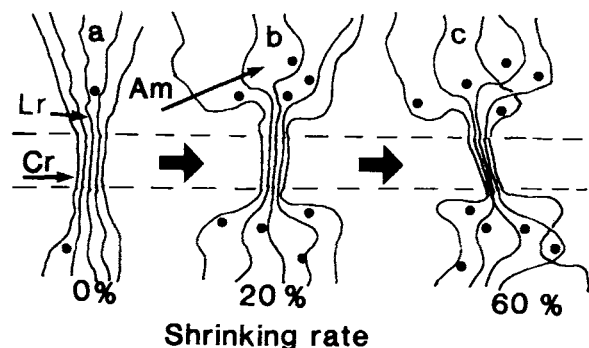


Figure 5 Schematic representation explaining the changes in the fine structure of silk fibroin fiber in the course of the shrinkage treatment and in the dyeability. Cr, crystalline region; Am, amorphous region; Lr, laterally ordered region; (●) dye molecules. (a) Untreated. Dye molecules tend to insert and attach within the amorphous region for the silk fiber with higher shrinkage (b,c) due to the disordering of the laterally ordered region and amorphous region.

in order to improve the dyeability of silk fibers. Table I lists the experimental results obtained by dyeing untreated and shrunk silk fibers at different temperatures (40, 60, and 80°C) with two different acid dyes, C.I. Acid Orange 7 and Acid Red 18. Both of them are anionic monoazo dyes, with one and three sulfonic groups, respectively, the latter having a molecular weight ($M_w = 534$) larger than the former ($M_w = 328$). The saturation values attained by silk fibers with 13% shrinkage were significantly larger than those of the untreated control sample for each temperature examined. It is interesting to note that at 40°C, the amount of dye absorbed by shrunk silk fibers was at least similar (C.I. Acid Red 18) or even larger (C.I. Acid Orange 7) than that of the untreated control sample at 80°C. The standard affinity, which measures the tendency of a dye to move from the dye bath onto the fiber substrate, increased slightly for shrunk silk fibers. Accordingly, the heat of dyeing increased, suggesting that a large number of reactive sites became available for the interaction between dye molecules and fibroin chains. The above findings are consistent with the hypothesis that the shrinking treatment is effective in increasing the dye uptake by inducing an apparent growth of the amorphous regions of the fiber. Thus, some reactive sites, partially or totally masked in untreated silk fibers for steric reasons,¹² become more accessible and can react with the dye molecules, resulting in a

net increase of dye uptake. Figure 5 shows a schematic representation of the changes in the fine structure of silk fibers following the shrinkage treatment, in relation to the changes in dyeing behavior. The laterally ordered region, connecting the crystalline with the totally amorphous regions in untreated silk fibers (a), are progressively disordered by the action of concentrated salt solution. This effect becomes evident even at relatively low shrinkage, below 20% (b), as shown by the behavior of the optical properties. Several amino acid residues located in these polypeptides sequences are free to interact with dyes by forming hydrogen bonds, electrostatic, and hydrophobic interaction, etc. A further increase of the extent of shrinkage can be effective in inducing other changes in the fine structure of silk fibers (c), such as a reduction of the crystal size. However, these additional effects should be considered almost completely unimportant from the point of view of the dyeing behavior, either for chemical or steric reasons. Therefore, a limited shrinkage can be successfully carried out in order to improve the dyeability of silk fibers by increasing the dye uptake, in response to specific technological requirements.

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