Structural characteristics and properties of *Bombyx mori* silk fiber obtained by different artificial forcibly silking speeds

Md. Majibur Rahman Khan, Hideaki Morikawa *, Yasuo Gotoh, Mikihiro Miura, Zha Ming, Yuji Sato, Masayuki Iwasa

Faculty of Textile Science and Technology, Shinshu University, Tokida 3-15-1, Ueda, Nagano 386-8567, Japan

Received 1 October 2007; received in revised form 6 December 2007; accepted 6 December 2007

Available online 24 January 2008

Abstract

To study the spinning condition of natural biopolymer silk, the silk fibers were directly acquired from *Bombyx mori* silkworm, N140 × C140 by a simple artificial forcibly silking method at the speed of 60, 120, 180 and 240 cm min⁻¹, respectively and its microstructure and physical properties were evaluated. The fine silk fibers (about 8 μm) were obtained at faster spinning speed, 240 cm min⁻¹. The tensile properties of silk fibers were remarkably increased with raising the forcibly spinning speeds. The β-sheet structure contents of silk fibers obtained at higher speed were considerably increased. The fibers obtained by different spinning speeds exhibited a fairly similar X-ray crystallinity, while the degree of molecular orientation increased with decreasing the fiber diameter. The fine silk fibers obtained at higher speed (240 cm min⁻¹) exhibited a slightly higher thermal stability, as shown by the upward shift of differential scanning calorimetry (DSC) decomposition temperature.

© 2007 Elsevier B.V. All rights reserved.

Keywords: *Bombyx mori* silk fiber; Artificial forcibly silking speed; Structure; Mechanical properties

1. Introduction

Silk fiber is one of the most familiar, as well as being one of very useful biopolymer, and the study of its structures, chemical, physical and mechanical properties has been extensively reviewed by various authors for thousands of years [1–3]. Several species of silk spinning insects exist in nature, including caterpillars and spiders. However, silk thread spun by the larvae of silkworm, *Bombyx mori* (B. mori) is of practical importance as a source of textile grade fibers. Among natural and synthetic fibers, silk occupies a leading position for its unique properties, such as water absorption, heat retention, handling, luster, comfort, brilliant color shades obtained by dyeing and printing, etc. [4]. Besides its use as a textile fiber, it has been considered as starting raw materials for non-textile applications, especially in the biomedical, cosmetic and biotechnological fields, such as surgical suture, wound cover materials, controlled drug release carriers, tissue engineering scaffolds and repair materials for skins, bones, ligaments, etc. [5].

The spun silk fibers consist of mainly two components, fibroin and sericin. Fibroin is the structural protein of silk fiber, whereas sericin is the water soluble proteinaceous glue that serves to bond the fibers together. The majority of fibroin’s composition is highly periodic, with simple repeating sections broken by more complex regions containing amino acids with bulkier side chains. The highly repetitive sections are composed of glycine (45%), alanine (30%), and serine (12%) in a roughly 3:2:1 ratio and dominated by [Gly-Ala-Gly-Ala-Gly-Ser]ₙ sequences. Fibroin is known to form mainly three kinds of conformations: silk I with a helical conformation, silk II with an anti-parallel β-sheet, and a random coil without definite orders. Sericin, which comprises approximately 25 wt% of the silkworm cocoon, contains glycine, serine, and aspartic acid totaling over 60% of the overall composition [6,7].

Cocoon is the only source of silkworm silk raw materials extensively used in the textile industry. Unfortunately, silk processing from cocoon to the finished clothing consists of a series of steps. Degumming is a key process during which sericin is removed by thermo-chemical treatment of the cocoon. Since degumming imposes a relatively harsh environment on the silk fibroin [8], the possibility of changes occurring in fibroin microstructure and mechanical properties, or even fibroin
degradation, must be considered. The effect of degumming on silkworm silk has been studied by micro-structural characterization techniques [9–11]. Recent investigation revealed that degumming process has been greatly influenced the tensile behavior, decrease in the initial elastic modulus and also changes the qualitative force–displacement curves of silk fibers [12,13]. Therefore, it is especially convenient to have the capability to obtain testable silk fibers without degumming a cocoon.

Many investigations have been made over the past decades on the artificial forcibly spinning of B. mori silk to overcome the difficulties of degumming, and especially to produce stronger silk fibers from silkworm silks [14–17]. Artificial forced spinning of silk from immobilized silkworms under steady and controlled conditions produces fibers that are superior to naturally spun ones [14]. The silkworm, like the spider, produces stronger fibers at faster spinning speeds [15]. So the production of silk fibers directly from silkworm before developing a cocoon is an extremely attractive and highly challenging objectives in the emerging field of biomimetics. However, until recently, there is no details investigation on the microstructure and physical properties of silkworm silk fibers obtained by forcibly silking.

The aim of this research was to study the spinning condition of B. mori silkworm silk fibers. Here we reported the structural characteristics and properties of silk fibers obtained by forcibly silking at different speeds.

2. Materials and methods

2.1. Materials

B. mori silkworms, N140 × C140, kindly supplied by Professor Rensuke Kanekatsu, Experimental Farm Laboratory, Shinshu University, Japan, were used in the experiment.

2.2. Forcibly silking process

The silkworms were reared to the fifth larval stage on a diet of mulberry leaves. Immediately after they stopped feeding subjected to constant surveillance to detect the onset of cocoon spinning. When silk was first observed, the worm was placed on a horizontal, flat, black vinyl surface and allowed to spin a short length of fiber. We select the plain and horizontal base so that the worms cannot construct cocoon, black base for easily detecting the silk, and vinyl surface to prevent silk from sticking. The schematic diagram of the forcibly silking method is shown in Fig. 1. At first, the spun fiber was grasped with tweezers and reeled from the silkworm by a silking device. The device consists of motor, speed controller, speed counter and a bobbin. Four acrylic sticks were folded on the different surface portion of the bobbin so that silk fibers cannot stick on it. The bobbin rotates and moves so that silk can wound on it. The speed controller controls the spinning speed, and speed rate is computed in the counter. The motor capacity of the device was from 10 to 600 cm min$^{-1}$. We tried to obtain silk fibers in as much as faster spinning speeds. After 240 cm min$^{-1}$, fibers were frequently broken and we could not able to obtain silks more than the above speed. Approximately 15 m of silk can be obtained in each process. Finally, the sufficient amounts of samples were collected in four different spinning speeds 60, 120, 180 and 240 cm min$^{-1}$, respectively. Fig. 2 shows the silk fiber storage system in every spinning stage. A strip of adhesive tape was fixed along an acrylic stick folded on bobbin and the fiber was cut along the stick, dividing the initial continuous fiber in samples of a length equal to the bobbin outer circumference (Fig. 2a). Then, one portion of the finer strips was firmly fixed on a plastic folder, and the fiber was unwrapped manually by a combination of rotation and translation perpendicular to the axis of the bobbin (Fig. 2b), taking care that the fibers remained parallel to each other until the strip that remained fixed on the bobbin stick was transferred and secured onto the plastic folder (Fig. 2c). By this method we could able to minimize experimental errors and obtained fibers in good position.

2.3. Experimental

Fourier transform infrared (FT-IR) spectroscopy was measured with a Shimadzu FT-IR-8400S infrared spectrometer by the ATR method in the region of 4000–400 cm$^{-1}$ at room temperature.
X-ray diffraction (XRD) photographs and wide angle X-ray diffraction (WAXD) profile were obtained by a Rigaku Rotorflex RU-200B diffractometer using Ni-filtered Cu Kα radiation generated at 40 kV and 150 mA. XRD photographs were developed using imaging plate.

The crystalline orientation of the fiber was determined quantitatively by Herman’s orientation factor generalized to a set of three crystallographic axes as follows:

$$f_x = \frac{3\langle \cos^2 \phi \rangle - 1}{2}$$

where $\langle \cos^2 \phi \rangle$ is the average value of square of the cosine of angle $\phi$ between the direction in the sample (fiber axis) and $x$-crystallographic axis. Assuming rotational symmetry about the fiber axis, the following equation was obtained:

$$\langle \cos^2 \phi \rangle = \frac{\int_0^{\pi/2} I(\phi) \cos^2 \phi \sin \phi \, d\phi}{\int_0^{\pi/2} I(\phi) \sin \phi \, d\phi}$$

where $I(\phi)$ is the intensity reflected from [h k l] planes, which are normal to the $x$-crystallographic direction. Here, $I(\phi)$ and $\phi$ were obtained from the azimuthal scan of the X-ray fiber diagram.

The tensile properties were measured with a Tensilon Model UTM-II-20 (Orientec Corporation), Japan using standard technique at 22 °C and 65% RH at a gauge length of 40 mm and strain rate of 100% min⁻¹. Tensile tests were carried out on several samples of silk collected from only one silkworm at different speeds to avoid the variability. Degumming treatment, such as, boiling in distilled water for 30 min greatly affects the tensile properties of forcibly reeled silkworm silk. It decreases yield strength and changes in the qualitative shape of force–displacement [13]. So we used the fibers directly obtained by forcibly silking. Special attention has been taken to collect fibers for mechanical test. In each spinning step, sufficient amount of specimen were obtained by pulling gently (as described in fiber storage system), taking care that they were not stressed during the process. The fibers were allowed to dry overnight in air before performing tensile test. Then the original fibers were cut into shorter length (about 60 mm) to prepare samples for tests. These are mounted across rectangular holes cut paper sheet supports to define the gauge length (40 mm) of mechanical test specimens. The both lower and upper ends (10 mm each) of fibers were glued to fix the fiber to the sheets. The adhesive was allowed to dry overnight before mechanical tests were started. The paper sheet was fixed in the tensile testing machines, and papers were cut along the discontinuous lines, so that the applied load can be transmitted through the fiber.

Prior to tensile strength measurement, silk fibers diameter was determined by optical microscope GS-551 (ONNO SOKKI LINEAR GAUGE SENSOR). Because of the pronounced variation of data, the diameters at 10 different positions of each specimen were measured to get the mean diameter.

Differential scanning calorimetry (DSC) measurement was performed by a Rigaku Denki Co. Ltd. instrumental (model DSC-8230) at a heating rate of 10°C min⁻¹ under N₂ gas atmosphere.

### 3. Results and discussion

#### 3.1. Silk diameter and tensile properties

The mean diameter including standard error bar of silk fibers obtained by forcibly silking at different speeds are shown in Fig. 3, and the statistical results of the diameter evenness variations are given in Table 1. The average diameter of silk was 13.5, 11.6, 10.6 and 8.0 μm in case of the spinning speed 60, 120, 180 and 240 cm min⁻¹, respectively. By obtaining silk fibers from
Table 1

<table>
<thead>
<tr>
<th>Spinning speed (cm min(^{-1}))</th>
<th>Mean fiber diameter (μm)</th>
<th>Maximum value of diameter (μm)</th>
<th>Minimum value of diameter (μm)</th>
<th>Standard deviation</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>13.5</td>
<td>24.5</td>
<td>8.0</td>
<td>3.85</td>
<td>0.44</td>
</tr>
<tr>
<td>120</td>
<td>11.6</td>
<td>16.8</td>
<td>7.4</td>
<td>3.19</td>
<td>0.38</td>
</tr>
<tr>
<td>180</td>
<td>10.6</td>
<td>16.6</td>
<td>6.5</td>
<td>2.26</td>
<td>0.27</td>
</tr>
<tr>
<td>240</td>
<td>8.0</td>
<td>15.7</td>
<td>5.7</td>
<td>1.90</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Silkworm cocoons with a special experimental procedure, it has been reported that the silk fibers diameter varies between from 15 to 19 μm, including the sericin coating [18]. In general, the faster the spinning speed, the thinner the silk diameter. Comparatively thinner silk fibers obtained in this experiment should be related to the faster forcibly spinning speed. It is observed that there is a pronounced scatter in the silk diameter along its length in case of every spinning stage though there is a decreasing tendency of variations with increasing spinning speed. At higher speed (240 cm min\(^{-1}\)) the fiber diameter was in the range of 5.7–15.7 μm, standard deviation was 1.90 and standard error was 0.24. The observed variation of silk fibers obtained at different speeds is attributed due to several reasons. The main reason is the change in the volume fractions of sericin and fibroin in silk with the progress of the spinning process as sericin were not removed from fibers in the experiment. A small measurement error may also exist though we measured the diameters at 10 different positions of each specimen in every spinning step to get the mean diameter.

The mean diameter of obtained silk in each spinning step was used to calculate its cross-sectional areas, assuming a circular cross-section. It has been reported that for the purpose of cross-sectional area measurement, fibers with an elliptical or oval cross-section can be treated as though the cross-section is circular. The maximum error is small if the axial ratio (length to width) of the cross-section is less than 1.5 [19]. Pérez-Rigueiro et al. (2000) showed that the shape anisotropy of silk fibers obtained from B. mori silkworm by forcibly silking is about 1.3, which is less than the maximum axial ratio/ovality of 1.5 [14]. Therefore, it has no significant loss of accuracy results from fitting the cross-section to a circle having as its diameter the average of the experimentally measured values. Fig. 4 shows the stress–strain curves of silk fibers obtained at different spinning speeds. It is observed that there was a noticeably increasing tendency of tensile strength with increasing speed. At 240 cm min\(^{-1}\), the obtained average tensile strength was about 650 MPa. A comparison study of tensile strength of B. mori silk fibers obtained by forcibly silking with those of raw silk.

![Stress-strain curves of silk fiber obtained by forcibly silking at different spinning speeds](image_url)
or un-forced spun silk reported in the literature is presented in Table 2. The strength of silk fibers obtained by forcibly silk- ing at faster speed is remarkably higher than those of naturally spun one. These results are consistent with the reports of forced silking previously cited in the literature [14,15]. From the above results, it is revealed that like spider silk, considerably higher strength silk fiber can be able to produce from B. mori silkworm silk by forcibly silking at faster spinning speed.

3.2. Crystalline structure and molecular orientation

FT-IR spectroscopy is a powerful technique to study structure at the molecular level, and reveals typical absorption bands sensitive to molecular conformation of silk proteins. A wealth of structural information can be obtained by analyzing the shape and position of bands in the amide I region of the spectrum. Selected amide I band frequencies have been correlated with the presence of α-helical, anti-parallel and parallel β-sheets and random coil structures [22–27]. In general, the amide I mode associated with the β-sheet conformation occurs in the range of 1618–1640 cm\(^{-1}\), the random coil conformations give bands in the range of 1640–1650 cm\(^{-1}\) and the α-helical conformation results in bands between 1650 and 1660 cm\(^{-1}\).

Fig. 5 shows FT-IR spectra of silk fibers obtained by forcibly silking at different spinning speeds. The spectrum of silk spec-

![Fig. 5. FT-IR-ATR spectra of silk fiber obtained by forcibly silking at different spinning speeds: (a) 60 cm min\(^{-1}\); (b) 120 cm min\(^{-1}\); (c) 180 cm min\(^{-1}\); (d) 240 cm min\(^{-1}\).](image)

imen obtained at nominal speed (60 cm min\(^{-1}\)) is characterized by the absorption bands at 1640 cm\(^{-1}\) (amide I), assigned to random coil conformation, 1515 cm\(^{-1}\) (amide II), attributed to the β-sheet structure, and 1230 cm\(^{-1}\) (amide III), random coil conformation [28–30]. On the other hand, with increasing the spinning speed the intensity of the absorption band of amide I gradually raised and shifted to 1618 cm\(^{-1}\) at the spinning speed of 240 cm min\(^{-1}\), in accordance with the structural changes into β-sheet conformation [29]. This result indicates that the β-sheet crystallization can be promoted by the changing of the artificial spinning speed to faster than those of silkworm spun silk

![Fig. 6. Deconvoluted FT-IR-ATR profiles of amide I of silk fibers obtained by forcibly silking at different speeds: (a) 60 cm min\(^{-1}\); (b) 240 cm min\(^{-1}\), which were shown in Fig. 5.](image)
fibers naturally. To clarify the secondary structure of silk fibers in details, the amide 1 profiles were deconvoluted using Gaussian function as shown in Fig. 6. The deconvoluted curves reveal that the random coil conformation (1647 cm$^{-1}$) is the most distinctive feature of fibers obtained at nominal speed, 60 cm min$^{-1}$. In the higher speed (240 cm min$^{-1}$), fibers exhibited a higher level of $\beta$-sheet conformations (1618 and 1700 cm$^{-1}$) and the $\alpha$-helical character (1660 cm$^{-1}$). In order to elucidate the structural features, XRD measurement was conducted.

X-ray diffraction has been often used to investigate the crystalline structure and molecular orientation of fiber materials. XRD images of silkworm silk spun at forcibly silking at different speeds are shown in Fig. 7. The patterns exhibit the typical pattern of a silk II crystal with high crystallinity and a high degree of crystallite orientation in every step of artificial spinning speed. To compare the degree of molecular orientation of fibers obtained at different speed, $\theta$ and an azimuthal scan were performed. The orientation coefficient was determined quantitatively based on the Herman’s orientation factor [31]. The crystalline orientation coefficients of the fibers obtained at different spinning speeds are illustrated in Fig. 8. The orientation coefficient values of the fibers obtained by forcibly silking were comparatively higher than those of natural cocoon fibers (degummed and raw silk), and an increasing tendency of the orientation coefficient was observed with increasing the forcibly spinning speed.

Fig. 7. X-ray photographs of silk fiber obtained by forcibly silking at different spinning speeds: (a) 60 cm min$^{-1}$; (b) 120 cm min$^{-1}$; (c) 180 cm min$^{-1}$; (d) 240 cm min$^{-1}$.

Fig. 8. Relationship between spinning speed of forcibly silking and the degree of crystallite orientation of silk fiber.
3.3. Thermal behavior

Fig. 9 shows the DSC thermograms of silk fibers obtained by forcibly silking at different spinning speeds. The obtained graphs are characterized by a broad endothermic transition attributed to the thermal decomposition of silk fibers with oriented β-sheet crystalline structure [32]. The shape and intensity of the endotherms did not change in relation to the fiber diameter variation. However, the peak temperature slightly moved upwards to the thermal decomposition of silk fibers with oriented β-sheet crystalline structure [32]. The shape and intensity of the endotherms did not change in relation to the fiber diameter variation. However, the peak temperature slightly moved upwards to the thermal decomposition of silk fibers with oriented β-sheet crystalline structure [32].

4. Conclusion

The silk fibers were obtained from a B. mori silkworm, N140 × C140 by a simple forcibly silking method at the speed of 60, 120, 180 and 240 cm min⁻¹, respectively and its structural characteristics and physical properties were evaluated. The fine silk fibers (about 8 μm) were obtained at faster spinning speed, 240 cm min⁻¹. The strength of silk fibers obtained by forcibly silking was higher than those of naturally spun one, and there was a remarkably increasing tendency of tensile strength with increasing spinning speed. At 240 cm min⁻¹, the average tensile strength of silk was about 650 MPa. The β-sheet structure of obtained silk fibers was greatly enhanced by increasing the silk spinning speed. The fibers obtained by different spinning speeds exhibited a fairly similar X-ray crystallinity, while the degree of molecular orientation increased with decreasing the fiber diameter. The high performance silk fibers with smaller diameters obtained by forcibly silking at faster spinning speed should be an attractive candidates for biomedical, electrical and textile applications, including tissue-engineered scaffolds, wound dressings and drug delivery systems.

Acknowledgements

This study was supported by a Grant-in-Aid for the Global COE Program by the Ministry of Education, Culture, Sports, Science and Technology of Japan. The authors wish to express their sincere thanks to Professor Rensuke Kanekatsu, Experimental Farm Laboratory, Shinshu University, Japan, for kindly supplying B. mori silkworms.

References

学霸图书馆

www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：

- 图书馆首页
- 文献云下载
- 图书馆入口
- 外文数据库大全
- 疑难文献辅助工具