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Acceleration effect of sericin on shear-induced β-transition of silk fibroin

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ABSTRACT

Although silk sericin (SS) occupies 25% of silk protein, its importance has often been overlooked in the natural silk spinning process and in the formation of the crystalline structure of silk fibroin (SF). In this study, we elucidated the role of SS in the crystallization process of SF under shear using SF/SS blend solutions. In order to apply shear stress to the blend solution, a rotating glass rod was inserted into a glass tube filled with the solution and the shear rate was determined to be in the range of 598–724 s^{-1}. After shearing, SF aggregates were formed and the amount of the aggregates increased with shearing time. Additionally, it was observed that the aggregate formation and β-sheet transition of SF were enhanced when a proper amount of SS was in the blend solution. Consequently, the SS considerably contributes to the structural transition of SF under shear. The SS can improve the shear-induced β-sheet transition and crystallization of SF.

1. Introduction

Silk fiber, which is mainly composed of the proteins, fibroin and sericin, has been an excellent textile material for a long time due to its outstanding mechanical properties. Recently, silk protein has received much interest as a biomaterial for its biological compatibility and functionality. Accordingly, there have been many attempts to create silk-based materials for specific purposes through various fabrication methods, e.g., gelation, spinning, film casting and foaming [1–9]. Nevertheless, despite efforts of many researchers, the regenerated silk protein materials exhibit poor physical and mechanical properties compared with natural silk. This is probably due to a lack of understanding of the nature of silk protein itself and the spinning process of the silk worm.

Due to its stable β-sheet structure, silk fibroin (SF) has high crystallinity as well as high molecular orientation and provides excellent tensile strength as the main component of silk fiber. Therefore, most studies are mainly focused on formation of the well-developed β-sheet structure of SF. Subsequently, it was discovered that such structural characteristics originated from the unique nature of SF and were regulated by many complicated parameters. So far a number of studies have tried to elucidate these parameters and the results can be summarized as follows:

1. Repetition of a regular amino acid sequence
2. Concentration process of fibroin solution in gland and solidification
3. Shear stress in gland and drawing process

Among these determinants, shear stress is critical for SF to transition from a random coil conformation to a β-sheet structure in aqueous solution. It is generally accepted that SF molecules aggregate and crystallize by themselves under shear condition without methanol treatment [10–12].

Silk sericin (SS), which occupies 25% of total weight of raw silk fiber, is mostly removed by a degumming process because it is believed that sericin plays a limited adhesive role of binding two fibroin strands and raw fibers in cocoons. Very few have focused on the important role of sericin in the fiber formation process of silk and in the structural transition of SF. Hence, the SF structure in nature and the silk regeneration process have so far been studied exclusive of sericin. But in more recent studies, it was reported that the crystallization and the mechanical property of the regenerated silk could be affected by SS. Lee observed a retardation of recrystallization of SF in an SF/SS blend by methanol treatment, suggesting that there is physical interaction between SF and SS molecules due to hydrogen bonds [13]. Such an effect of SS may be due to SS' hydrophilicity and similar result has been reported that

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the β-sheet transition of SF can be achieved by blending with hydrophilic polymer, such as hyaluronic acid, without any general crystallization process, methanol treatment or shearing [14]. We also found that the crystallinity and tensile strength of the regenerated silk filament, fabricated from partially degummed cocoons (containing ca. 10% SS), were improved [5]. These reports indicate that SS seems to play an important role in the structure and mechanical property of SF by affecting the conformational transition of SF molecules.

From our preliminary study, it was observed that the mechanical property of regenerated silk film cast from sheared SF/SS blend solutions was improved while the crystallinity and the mechanical property of SF/SS blend film prepared from unsheared solutions were hardly changed compared with those of pure SF film. And it was confirmed that the shear condition might play a key role in the β-sheet transition of SF in an SF/SS blend. Therefore, we discuss herein the role of sericin in the formation of SF crystalline structure in an SF/SS blend, focusing especially on the β-sheet transition.

2. Experimental section

2.1. Materials

In order to obtain pure SF, cocoons from *Bombyx mori* were boiled in an aqueous solution of 0.3% (w/v) sodium oleate and 0.2% (w/v) sodium carbonate for an hour and subsequently washed with warm distilled water several times, followed by drying at 60 °C. The degummed cocoons were then dissolved in 9.3 M LiBr aqueous solution for an hour at room temperature. The solution was filtered and dialyzed against distilled water using a cellulose tube (MWCO: 12–14 kDa) for 3 days. Then the aqueous SF solution was freeze-dried and eventually, the regenerated SF sponge was obtained. On the other hand, SS was extracted from the cocoons in 8 M urea solution for an hour at 80 °C. Then the solution was filtered and dialyzed following the same protocol as for SF solution. The SS solution was turned into a weak gel during the dialysis and finally the SS gel was freeze-dried.

The SF/SS blend solution was prepared by simultaneously dissolving the regenerated SF and SS at appropriate weight ratios in 9.3 M LiBr solution for an hour at room temperature. The mixing ratios of SF and SS were 100:0, 90:10, 80:20 and 75:25, and each sample was designated as SFSS1000, SFSS9010, SFSS8020 and SFSS7525, respectively. Then the completely dissolved SF/SS blend solution of 10% (w/v) was dialyzed for 3 days to remove salt. After the dialysis, the solutions of varying SF/SS blend ratios were diluted to concentrations in the range of 3–4.5 wt%. Hence, each solution was diluted or concentrated to the same concentration for measurements. To concentrate a solution, the dialyzed SF/SS solution was poured into a cellulose tube (MWCO: 3500 Da) and the tube was immersed in 50% (w/v) polyethylene glycol (M_w: 20,000) aqueous solution for appropriate times (approximately 8–12 h) at 4 °C to prevent denaturation by warm temperature or shearing.

The regeneration processes of SF and SS used in the experiments do not cause severe damage and hardly decrease the molecular weights [15–18]. Our preliminary study confirmed that the molecular weights of regenerated SF and extracted SS were not different from what has been reported when measured by electrophoresis (data not shown).

2.2. Shearing condition

For shearing, the concentrations of SF/SS blend solutions were adjusted to 5 wt% and each solution of 2 ml was moved into a glass which has an 11 mm inner diameter and 15 cm depth. Then a glass rod of 10 mm diameter was placed in the tube center. The length of the glass rod was 30 cm. The upper part was fixed on the spin motor's shaft and the opposite end located above 5 mm from the bottom of the glass tube. The gap between the tube wall and the rod was 0.5 mm. The glass rod was locked by a spin rate controllable motor and the spin rate was kept at 600 rpm (62.8 rad/s). In this condition, the shear rate was calculated by Eq. (1) [11]:

$$\dot{\gamma}(r) = \frac{2\Omega}{r^2\left(\frac{1}{R_1^2} - \frac{1}{R_2^2}\right)} \tag{1}$$

where r is the radius from the rotation axis of the rod ($R_1 \le r \le R_2$), Ω is angular frequency (rad/s), R_1 is the radius of the glass rod and R_2 is the inner radius of the glass tube. The calculated value of the shear rate varied from 598 s^{-1} at the surface of the glass rod to 724 s^{-1} at the tube wall. All shearing was performed at room temperature.

2.3. Shear viscosity measurement

The shear viscosities of the SF/SS blend solutions were measured using Rheometric Expansion System (ARES, Rheometric Scientific, US) as a function of shear rate (0.01–1000 s^{-1}). A cone and plate geometry were used to ensure a constant shear rate. The radius and angle of the cone were 50 mm and 0.04 rad, respectively. The measurement was conducted at room temperature.

2.4. Dynamic light scattering (DLS)

To measure the molecular size and distribution of SF and SS in aqueous solution before and after shearing, DLS analysis was conducted using a DLS spectrophotometer (DLS-7000, Otsuka Electronics, Japan). In order to prepare the solutions, the 5 wt% SF/SS blend solutions were sheared for 30 min; after shearing, the concentrations were diluted to 1 wt%. The concentration of the unsheared solution was also adjusted to 1 wt%. All solutions were kept at 4 °C until measurements were taken to prevent aggregation of SFs. Each solution was moved into a cell of 1 cm path length and an Argon laser was used as a light source. The measuring angle of the scattering was 90° and all measurements were conducted at room temperature. The hydrodynamic radius and distribution were calculated by analysis software.

2.5. Circular dichroism (CD)

The samples were prepared for CD analysis by the same method as described above for DLS measurements but the concentrations were adjusted to 0.05 wt% instead of 1 wt%. The CD spectra of the SF/SS blend solutions were obtained using a circular dichroism detector (J-715, Jasco, Japan) and the wavelength range was 190–250 nm with a resolution of 0.2 nm and an accumulation of 20 scans at a scanning rate of 50 nm/min. In order to estimate the β-sheet fraction of the solution, the normalized ellipticity at 217 nm was obtained by Eq. (2) [19,20].

$$f_\beta = \frac{[\theta]_{217}^{\exp} - [\theta]_{217}^{\mathrm{coil}}}{[\theta]_{217}^{\max} - [\theta]_{217}^{\mathrm{coil}}} \tag{2}$$

where f_β is the fraction of β-sheet, $[\theta]_{217}^{\exp}$ is the measured value of the ellipticity at 217 nm, $[\theta]_{217}^{\mathrm{coil}}$ is the ellipticity at 217 nm of a spectrum corresponding to SF's random coil conformation (minimum value among the results) and $[\theta]_{217}^{\max}$ is the maximum ellipticity value, which is expected for the sample with the highest β-sheet content.