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Crystallization of silk fibroin from solution

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Abstract

X-ray diffraction and differential scanning calorimetry (DSC) were carried out to clarify the crystallization mechanism of silk fibroin from liquid silk (native silk fibroin solution). In the course of the evaporation of water from liquid silk, silk fibroin crystallizes when weight loss of water reaches 40% at the temperatures below 120°C. The crystallization occurs 20 min after the specimens are put at above 100°C and 1–3 h at below 100°C.

In the DSC curve of liquid silk of domestic silk *Bombyx mori*, the α–β transition occurs at 51°C. The crystallization temperatures of wild silk *Antheraea yamamai*, *Antheraea pernyi* and *Dictyoploa japonica* are 59, 67 and 62.5°C, respectively. The exothermic DSC peaks at these temperatures are attributed to the conformational change to the β-form.

Keywords: Silk fibroin; DSC; Crystallization; α- to β-form transition; Wild silk

1. Introduction

Silk fibroin has a long history of use in the production of fashionable and comfortable fabrics for apparel. Fiber features, such as luster and fineness, result from the fiber formation process [1]. To clarify the mechanism of silk fiber formation, the crystallization process should be investigated by the thermal analysis using liquid silk and silk fibroin solutions.

Shimizu [2] and Kratky and Schauenstein [3] studied dried silk fibroin obtained from the silk gland of matured silkworm of *Bombyx mori* by X-ray diffraction and obtained two different crystal forms, the α-form (or silk fibroin I) [4] and the β-form (or silk fibroin II) [5].

However, no detailed studies have been made on the crystallization behavior and thermal properties of silk fibroin. Therefore, liquid silk fibroin was obtained from the silk gland of matured silkworm and the crystallization process of silk fibroin was studied by thermal analysis and X-ray diffraction in aqueous

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solutions and films to clarify the mechanism of silk fiber formation.

The crystallization process of liquid silk from the domestic silkworm, *B. mori* and the wild silkmoths: *A. yamamai*, *A. pernyi* and *D. japonica* was compared using a heat-leakage scanning calorimeter.

2. Experimental

Liquid silk fibroin was obtained from the silk gland of full-grown larvae *B. mori* 1 day prior to spinning. The silk glands were removed and thoroughly rinsed with deionized water to eliminate sericin. They were then cut into three distinct divisions: anterior, middle and posterior divisions. Each division was collected and placed in water.

Liquid silk containing 25% fibroin was prepared from the posterior part of middle division of the *B. mori* silkworm while the liquid silks for *A. yamamai*, *A. pernyi* and *D. japonica* were prepared from the posterior divisions. Aliquots of solution were placed on glass slides and then dried at constant temperatures of 20, 35, 40, 45, 60, 80, 100, 120, 140 and 160°C, respectively.

Thermal analysis was carried out using a heat-leakage scanning calorimeter SEIKO Model SSC-100. X-ray diffraction patterns were obtained by a Rigaku Type 4042 X-ray diffraction apparatus operated at 35 kV and 20 mA.

3. Results and discussion

Fig. 1 shows the X-ray diffraction patterns of silk fibroin of *B. mori* dried at different drying conditions.

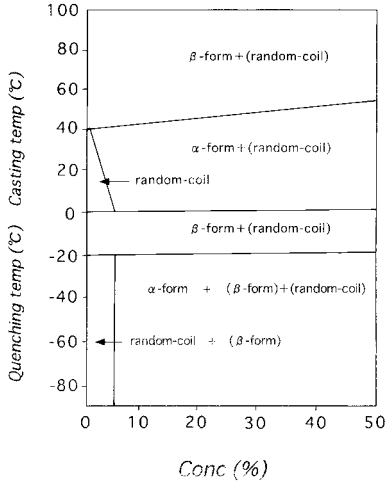

Fig. 2. The relation between conformation, quenching or casting temperature and concentration of silk fibroin, *B. mori*.

Silk fibroin was crystallized as the water evaporated during the process of drying. The crystal structure was changed from the α- to β-form depending on the drying temperature and initial silk fibroin concentration in water.

The α- and β-form crystals can be obtained by casting of a concentrated silk fibroin solution at temperatures of 0–40 and 50°C or above, respectively, whereas casting of a dilute solution at 0–40°C results in amorphous films with a random coil conformation as shown in Fig. 2.

When the aqueous solution of silk fibroin was cooled below 0°C, the crystal structure contained both the α- and β-form. The β-form is generally observed in drawn samples of silk fibroin. When the water in the solution was frozen below 0°C, the silk fibroin was subjected to shear and crystallization.

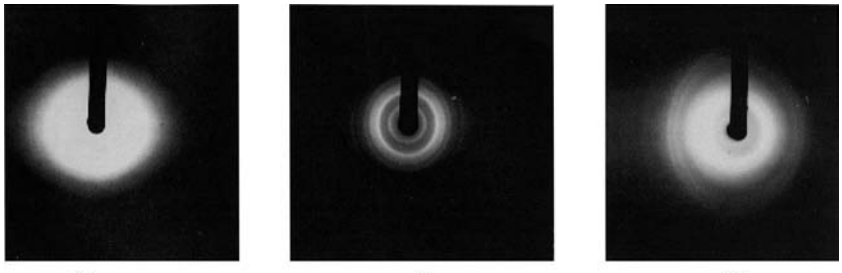

Fig. 1. X-ray diffraction patterns of silk fibroin, *B. mori*: (a) amorphous, (b) α-form (silk fibroin I) and (c) β-form (silk fibroin II).

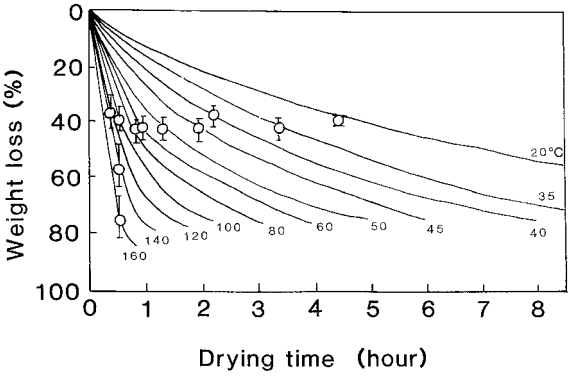

Fig. 3. Weight loss of water vs. drying time of liquid silk at various temperatures. (Φ): Starting time of crystallization.

Liquid silk fibroin was dried at various temperatures and the weight loss during the drying and the starting time of crystallization are plotted in Fig. 3. Liquid silk fibroins dried at 20, 35, 40 and 45°C were dried slowly and crystallization began to start after 4.5, 3.5, 2.25 and 2.0 h, respectively. When the drying temperatures were in the range of 50–80°C, crystallization started within 1.5 h and starting time of crystallization gradually became shorter with higher temperatures. Above 100°C, crystallization began to start between 20 and 30 min of drying. Higher drying temperatures did not shorten starting time of crystallization. At drying temperatures of higher than 120°C, the weight decreased by more than 75% (liquid silk contains 75% water), some of the silk fibroin might be decomposed at the higher temperatures.

The weight loss of water at starting of crystallization is plotted against drying temperature in Fig. 4. For convenience the concentration of silk fibroin in water is plotted on the ordinate. At the drying temperature of lower than 120°C, crystallization started when the concentration of silk fibroin became 60–70%. When the drying temperature was above 140°C, the specimen became yellowish and there was a marked decrease in weight, indicating degradation of a part of the specimen. This may cause the apparent rise of the concentration of fibroin at the starting time of crystallization.

Fig. 5 shows the degree of crystallinity of liquid silk fibroin as a function of drying temperature. The degree of crystallinity obtained by the density method was as high as 33–43%, reaching a minimum around 40–45°C. A little lower value was obtained from the X-ray method. However, similar tendency was observed in the degree of crystallinity determined by the X-ray method. The molecular form of silk fibroin is unstable in the temperature range of the α–β form transition (Fig. 2) and the crystallization is inhibited. Above a drying temperature of 60°C, many voids occur in the specimen, causing an apparently low degree of crystallinity as measured by the density method. In fact, many voids were observed in the specimen under a light microscope.

Fig. 6 shows the crystallization time, for the crystallization to be completed, vs. the drying temperature.

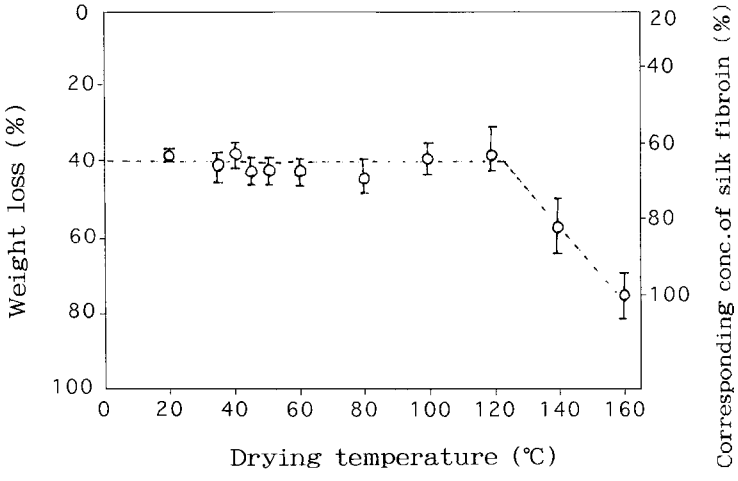

Fig. 4. Weight loss of water vs. drying temperature of liquid silk.

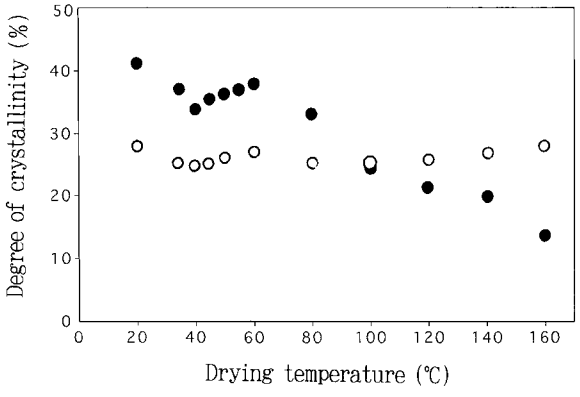

Fig. 5. Degree of crystallinity vs. drying temperature of liquid silk. (●): Density method; (○): X-ray method.

The result is best described by two straight lines, one for drying temperatures of 20–45°C and another for temperatures above 50°C, which intersects at about 48°C. This fact can be related to two different crystallization mechanisms. The first line corresponds to the α-form, which requires a longer crystallization time, and the second line to the β-form with the shorter crystallization times. The intersection of these two lines correspond to the α- to β-form transition, which occurs around this temperature.

When an aqueous solution of fibroin was frozen at the temperature range of 0 and −196°C by quenching prior to drying, β-form crystals are obtained for temperatures ranging from 0 to 20°C irrespective of the concentration of fibroin. On the other hand, when the solution is quenched below −20°C, both the β-form and random coil conformation are obtained, depending on the initial concentration. Fibroin molecules in dilute aqueous solution are in the random coil conformation. Therefore, extremely rapid freezing of a very dilute solution does not permit the change to the β-form to occur and the random coil conformation in a dilute solution is maintained in the film. These results indicate a relationship between conformations. A phase diagram was constructed from these data as shown in Fig. 2, which is more precise than the phase diagram we reported before [6].

In the DSC curve of liquid silk of *B. mori* (Fig. 7), an endothermic peak starts at 51°C and has the maximum at 60.5°C. When temperature was raised above 60.5°C, the liquid silk changed from transparent to yellowish and became slightly turbid. Fibroin molecules of *B. mori* assume the random coil conformation in aqueous solution. It has been reported that the random coil conformation is converted to the α-form on treatment with water below 60°C and to mixtures of the α- and β-form conformations above 70°C [2]. Therefore, the endothermic peak at 60.5°C is attributed to the random coil to β-form transition.

Fig. 8 shows the DSC curves of liquid silk from *A. yamamai*, *A. pernyi* and *D. japonica* recorded at a heating rate of 1.2 K min^{-1}. In the DSC curves of *A. yamamai* and *A. pernyi* exothermic peaks were observed beginning at 62.5 and 67°C and having a maximum at 65 and 70°C, respectively. When the temperature exceeded the exothermic peak tempera-

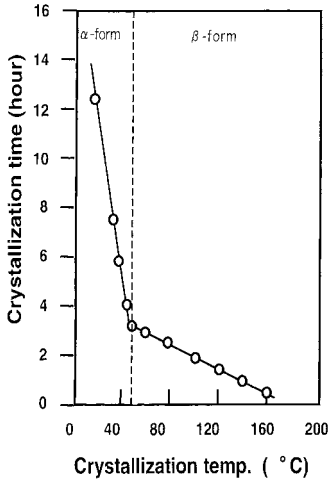

Fig. 6. Crystallization time, for the crystallization to be completed, vs. drying temperature of liquid silk.

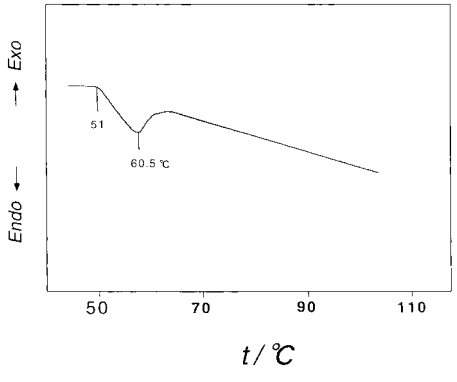

Fig. 7. DSC heating curves of liquid silk of domestic silkworm, *B. mori*.

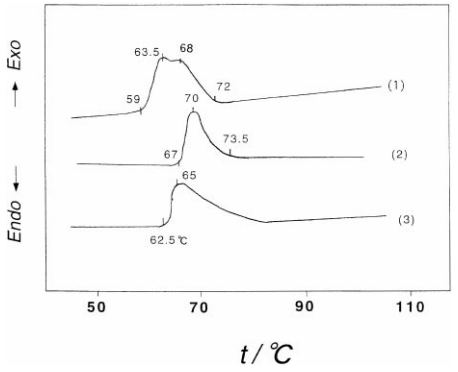

Fig. 8. DSC heating curves of liquid silk of wild silkworms: (a) *D. japonica*, (b) *A. yamamai* and (c) *A. pernyi*.

ture, liquid silks from *A. yamamai* and *A. pernyi* became cloudy and finely dispersed precipitates were observed. From the X-ray diffraction pattern the precipitates were crystalline and had the β-form conformations. The conformation of *A. yamamai* fibroin in liquid silk has been reported to be the α-helix [7]. Therefore, the exothermic peak at 65°C is attributed to formation of the β-form crystals accompanying the α-helix to β-form transition [8]. In the DSC curves of *D. japonica*, two exothermic peaks were observed at around 63.5 and 67°C. The peaks were attributed to the conformational change of β-form.

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