International Journal of Biological Macromolecules 75 (2015) 398–401

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Influence factors analysis on the formation of silk I structure

Jinfa Ming[a,b], Fukui Pan[a], Baoqi Zuo[b,*]

[a] The College of Textiles & Fashion, Qingdao University, Qingdao 266071, China
[b] National Engineering Laboratory for Modern Silk, College of Textile and Clothing Engineering, Soochow University, Suzhou 215123, China

ARTICLE INFO

Article history:
Received 26 September 2014
Received in revised form 2 February 2015
Accepted 3 February 2015
Available online 9 February 2015

Keywords:
Silk fibroin
Silk I
Temperature–concentration–humidity

ABSTRACT

Regenerated silk fibroin aqueous solution was used to study the crystalline structure of *Bombyx mori* silk fibroin in vitro. By controlling environmental conditions and concentration of silk fibroin solution, it provided a means for the direct preparing silk I structure and understanding the details of silk fibroin molecules interactions in formation process. In this study, silk fibroin molecules were assembled to form random coil at low concentration of solution and then, as the concentration increases, were converted to silk I at 55% relative humidity (RH). At the same time, the structure of silk fibroin forming below 45 °C was mostly in silk I. A partial ternary phase diagram of temperature–humidity–concentration was constructed based on the results. The results showed silk I structure could be controlled by adjusting the external environmental conditions. The enhanced control over silk I structure, as embodied in phase diagram, could potentially be utilized to understand the molecular chain conformation of silk I in further research work.

1. Introduction

Many types of silk have been characterized, including silk fibroin (SF) from *Bombyx mori*, tussah silk, and dragline silk from the spider *Nephila clavipes* [1–3]. *Bombyx mori* silk contains two main proteins, fibroin and sericin. Fibroin is the structural protein, whereas sericin is the water-soluble glue like protein surrounding fibroin protein [4,5]. SF consists of two macromolecules, with molecular weights of 390 and 25 kDa for heavy and light chains, respectively [6,7]. The crystal regions of heavy chain are dominated by the hydrophobic sequence Gly-Ala-Gly-Ala-Gly-Ser (GAGAGS) amino acid motif [7,8].

Crystalline structure in SF has been an active topic of study for several decades [9]. The structure of two crystalline forms in SF, silk I and silk II, has been reported based on all kinds of experimental results [10–13]. The silk II form is characterized by an anti-parallel β-sheet structure [11,14]. However, the less stable silk I form has remained poorly understand. At present, many investigations of the structure of silk I form have been studied [15–18]. These studies indicate that the conformation of silk I chain is a repeated β-turn type II that is capable of forming intra-molecular hydrogen bonds [19]. At the same time, many models with limited experimental data are proposed to describe the structure of silk I, such as crankshaft model [10], etc.

Thus, in this present study, in order to better research the structure of silk I, the important thing is how to prepare the stable silk I structure using regenerated SF aqueous solution at room temperature. At our previous works, SF aqueous solutions were slowly concentrated to form silk I at stable 55% relative humidity (RH) [6]. This study was focused on elucidation of influence factors such as drying time, concentration, and temperature, etc. in controlling the forming process of silk I. At the same time, the role of water in silk I formation process was also discussed in detail.

2. Experimental

2.1. Materials

Bombyx mori silks were bought from Zhejiang province, China. All chemicals (sodium carbonate, lithium bromide, ethanol, polyethylene glycol (PEG), etc.) were analytical grade from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China), and also used without further purification.

2.2. Preparation of SF solutions

Raw *Bombyx mori* silks were boiled for 30 min in aqueous solution of 0.02 M Na_2CO_3 and then rinsed thoroughly with distilled water to extract the sericin proteins. After drying, degummed

* Corresponding author. Tel.: +86 512 67061157; fax: +86 512 67246786.
E-mail addresses: jinfa.ming@gmail.com (J. Ming), mingjinfa@qdu.edu.cn (F. Pan), bqzuo@suda.edu.cn (B. Zuo).

http://dx.doi.org/10.1016/j.ijbiomac.2015.02.002
0141-8130/

silk fibers were dissolved in 9.3 M LiBr solution at 60 °C for 4 h, yielding a 10% (w/v) solution. This solution was dialyzed against distilled water using Slide-A-Lyzer dialysis cassettes (Sigma, USA, molecular weight cut-off 3500) for 3 days to remove the salt. The concentration of aqueous silk solution was ~3.0 wt%, determined by weighting the remaining solid after drying. And then, SF aqueous solution (3.0 wt%, 50 ml) was dialyzed against 20 wt% PEG (10,000 g/mol) solution at 5 °C by using Slide-A-Lyzer dialysis cassettes (MWCO 3500). The volume ratio of PEG to SF solution was 100:1. By osmotic stress, water molecules in SF solution moved into the PEG solution through the dialysis membrane obtaining higher concentrated solution. After the required time, the concentrated SF solution was collected by syringe to avoid excessive shear and the concentration (about 10.0 wt%) was determined by weighting the remaining solid after drying. Other concentration solution was obtained through diluted 10.0 wt% SF solution using distilled water.

2.3. Atomic force microscopy (AFM)

The morphology of SF aqueous solution in silk I formation process was observed by AFM (Veeco, CA) in air. A 225 μm long silicon cantilever with a spring constant of 3 N m^{-1} was used in tapping mode. For clearly observation, different time points of SF solution in silk I formation process were diluted to 1.0×10^6 to disperse SF molecules with deionized water. Once diluted, 1 μL diluted SF solution was quickly dropped onto fleshly silica surface and dried under nitrogen gas.

2.4. X-ray diffraction

To analyse the crystalline structure of SF films obtained from silk I formation process, X-ray diffraction (XRD) experiments were measured on X Pert-Pro MPD (PANalytical, Netherlands) with Cu Kα radiation working at 40 kV and 40 mA in the interval range from 5° to 45° with a scan rate 2° min^{-1}. The incident beam wavelength was 0.154 nm. The intensity was finally corrected for changes in the incident beam intensity, sample absorption, and background.

2.5. Mechanical properties

SF films with different crystalline forms (random coil, silk I, and silk II) were cut into 50 mm × 5 mm rectangles with thickness of 70–150 μm. The thickness of these films was measured using a micrometer. Before using an automatic tensile tester (model 3365 electronic strength tester, Instron, Boston, USA) to characterize the mechanical properties of these films, these samples were kept for 24 h at standard atmospheric conditions (20 °C, 65%RH). During test process, distances between grips and test speeds were set to 20 and 10 mm min^{-1}, respectively. At the same time, the pre-tension was 0.2 cN. An average of five measurements was reported as the mean ± standard deviation for each sample.

3. Results

3.1. Effect of drying times on silk I formation

In our previous study, silk I structure was directly formed when the environment humidity was 55% [6]. Therefore, this paper used SF aqueous solution (2.0 wt%) to study drying times affecting the formation of silk I. Fig. 1 showed XRD data of SF films preparing for different drying times at certain conditions (20 °C, 55%RH). After drying 1 d, Fig. 1a depicted the typical random coil of SF, characterized by the presence of a broad peak in the diffraction angle range from 5° to 45°. When the drying time was 2 d, the main diffraction peaks were also located at 20.4°, attributing to random coil structure (Fig. 1b). However, increasing drying times more than 3 d, the main diffraction peaks were appeared at 12.4°, 20.1°, 24.5°, 27.9°, and 37.1°, corresponding to the crystalline spacing of 0.75 (I), 0.46 (I), 0.39 (I), 0.33 (I), and 0.26 (I) nm, respectively, attributing to typical silk I (Fig. 1c and d) [6,15].

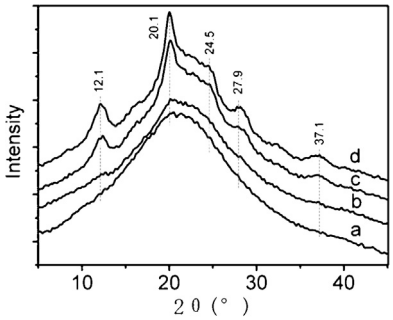

Fig. 1. Effect of drying times on the conformation formation of silk I; the drying times were as follows: (a) 1 d, (b) 2 d, (c) 3.5 d, and (d) 5 d, respectively.

3.2. Effect of SF concentration on silk I formation

In order to study the SF concentration affecting the silk I structure formation, this experiment was used equal solution (35 g) to study the SF structure formation at constant environmental condition (20 °C, 55%RH). Fig. 2 depicted XRD results of SF films forming at different concentrations. Fig. 2a and b exhibited the typical random coil structure, characterized by the presence of a broad peak in the diffraction angle range from 5° to 45° at solution with lower concentrations (0.5 and 1.0 wt%). With the increase of SF concentration, Fig. 2c showed the diffraction peaks at 12.3° and 20.1° at 3.0 wt% concentrations, attributing to silk I (β-turn) and silk II (β-sheet), respectively. At the same time, the diffraction peaks at 12.3°, 24.5°, 28.3°, 32.7°, and 36.9° were observed, when SF concentration was more than 7.4 wt% (Fig. 2d and e). These peaks were attributed to typical silk I structure. Therefore, SF molecules were assembled to form random coil at low concentration in solution and then, as the concentration increases, were converted to silk I.

3.3. Effect of temperature on silk I formation

As known, SF molecules with different structures were formed in aqueous solution by self-assembly. In self-assembly process, the drying rate of aqueous solution was strongly correlated with the self-assembly rate of SF, which was decided by environmental temperatures. Tsukada studied effect of drying rate on the structure of tussah silk fibroin cast from aqueous solutions at different temperatures [20]. The results showed the drying rate played an important role in determining the molecular conformation taken by silk on drying [21]. At low drying rate, α-helix was formed.

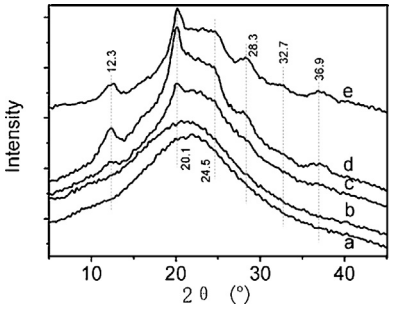

Fig. 2. Effect of SF concentration on the formation of silk I; the concentrations of SF aqueous solution were as follows: (a) 0.5 wt%, (b) 1.0 wt%, (c) 3.0 wt%, (d) 7.4 wt%, and (e) 10.0 wt%, respectively.