International Journal of Biological Macromolecules 119 (2018) 821–832

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Effect of molecular weight on the structure and mechanical properties of silk sericin gel, film, and sponge

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ARTICLE INFO

Article history:
Received 12 April 2018
Received in revised form 30 July 2018
Accepted 1 August 2018
Available online 03 August 2018

Keywords:
Sericin
Molecular weight
Structural characteristics

ABSTRACT

In this study, the effect of the MW on the structure and properties of sericin film, sponge, and gel was examined. As the MW of sericin increased, the gelation of the sericin aqueous solution was found to be accelerated, and the gel strength, and the gel-sol transition temperature increased. Irrespective of the casting solvent (water and formic acid) and form of sericin (gel, film, or sponge), the crystallization of the sericins was accelerated. The mechanical properties of the sericin sponge were remarkably improved upon increasing the MW of sericin. The MW of sericin almost did not have an effect on the cell toxicity. As the MW of sericin is increased, the sericin sponge becomes denser and its porosity is reduced, leading to a decrease in the swelling ratio. These results indicate that various characteristics of the sericin forms can be modulated by controlling the MW of sericin, with enhanced potential for biomedical and cosmetic applications.

1. Introduction

Raw silk is a natural composite fiber consisting of fibroin and sericin. Sericin binds and covers the two fibroin filaments in raw silk. When sericin is removed from raw silk, the two fibroin filaments are uncovered rendering the degummed silk fiber much finer and thereby improving the feel of silk textile. Further, the regular triangular shape of the fibroin filaments remarkably enhances the luster of the degummed silk fiber compared to that of raw silk. Based on these reasons, sericin is removed to improve the value of silk as a textile fiber and it has been considered a useless material.

However, recently, reports regarding new functions of sericin are changing this prejudice. That is, sericin is biodegradable [1], has high UV [2] and oxidation resistance [3], high water retention [4], and hypoglycemic effects [5]. Moreover, it inhibits UV-B-induced apoptosis in human skin keratinocyte [6], lowers cholesterol [7,8], and heals wounds [9,10]. These useful properties have accelerated the studies related to the biomedical, cosmetic, and functional health food applications of sericin [11–13].

For these applications, sericin should be fabricated in various forms including gel [14–17], film [16–20], sponge [17,21,22], particle [23–25], and fiber [26]. Therefore, studies on the fabrication and properties of these sericin forms are being conducted extensively. One of the main drawbacks of the sericin forms for various applications is their poor mechanical properties. Therefore, many studies have been performed to improve their mechanical properties. However, researchers have chosen easier and simpler methods to improve the mechanical properties. For example, the inclusion of additives such as glycerin, sorbitol [27], poloxamer [28], glycerol, and graphene oxide [29] in sericin. Although this is an easy approach, it is not very effective because the use of additive might negatively affect the unique properties of sericin and make the biomedical applications of sericin more difficult.

Before the incorporation of a foreign material, it is necessary to find a way to obtain the best performance of sericin itself. In other words, as the mechanical properties of sericin forms are affected by the preparation conditions, it is necessary to understand the relationship between them, and the optimum fabrication condition yielding the best-performing sericin should be found before including an additive.

In this context, basic studies on sericin with respect to the effect of preparation conditions on the structural characteristics and properties of different sericin forms have been conducted by our group. The fabrication of sericin forms includes the following considerations: The casting solvent [19], sericin concentration [17,19], ethanol addition [17],

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https://doi.org/10.1016/j.ijbiomac.2018.08.006
0141-8130/

centrifugation [30], storage and drying temperatures [16], and silkworm variety [31].

The molecular weight (MW) of silk proteins is one of the important factors affecting their properties. Therefore, the effect of the MW on the properties of the silk fibroin (SF) solution [32], wet-spun fiber [33,34], electro-spun web [33,35], and gelation [36,37] has been extensively studied. Moreover, sericin samples with various MWs can be obtained by different extraction conditions and the related studies have been conducted [38–40]. However, the effect of the MW on the structural characteristics and properties of sericin gel, film, and sponge has not been studied in detail, yet. Therefore, in the present study, sericin samples with different MWs were extracted and the effect of the MW on the structural characteristics and properties of the obtained sericin samples was examined in detail.

2. Experimental

2.1. Preparation of an aqueous solution of silk sericin

To extract silk sericin, *Bombyx mori* silk cocoons were treated with hot water (120 °C) using an autoclave (JSAC-60, JSR, Japan) [16,17]. As shown in Table 1, different extraction conditions including the treatment time and liquor ratio were used to extract four sericin samples (SS-10, SS-30, SS-30-30, and SS-30-90) with different MWs. SS-10 and SS-30 were extracted by treating the silk cocoons with hot water (120 °C) for 10 min and 30 min, respectively. SS-30-30 and SS-30-90 were obtained by a two-step extraction process. That is, the silk cocoons were treated with hot water for 30 min. Then, the extracted sericin aqueous solutions were treated once again at 120 °C for 30 min or 90 min using the autoclave to obtain SS-30-30 or SS-30-90 aqueous solutions, respectively.

After each extraction, the sericin aqueous solutions were filtered through a non-woven fabric. As a result, 2.0 and 0.6% (w/w) SS-10 sericin aqueous solutions were obtained from liquor ratios of 1:10 and 1:25, respectively. For other samples with other MWs of sericin (SS-30, SS-H30-30, SS-30-90), 2.5 and 1.0% (w/w) sericin aqueous solutions were prepared from liquor ratios of 1:10 and 1:25, respectively. Lower concentrations of SS-10 solutions (2.0 and 0.6%) than other solutions (2.5 and 1.0%) are due to the less extraction time (10 min) than others (30 min) To prepare the same concentrations of the sericin aqueous solutions, the 2.0 and 0.6% (w/w) SS-10 solutions were concentrated to 2.5 and 1.0% (w/w) solution by evaporating them at 80 °C for 20 min using a rotary evaporator (N-1200A, EYELA, Japan). The sericin concentration in the aqueous sericin solutions was measured using a moisture analyzer (XM60, Precisa Gravimetrics, Dietikon, Switzerland). To obtain sericin powders, the aqueous solutions of sericin samples were poured into Petri dishes and solidified at 80 °C in a drying oven.

2.2. Fabrication of sericin gel, film, and sponge

The 2.5% (w/w) sericin aqueous solutions with different MWs of sericin were stored at 4 °C for different periods (0–7 days) to examine their gelation behaviors. To investigate the molecular conformation of sericin gels stored for different periods, each of the gels prepared by storing the sericin aqueous solution for 1 day and 10 days were dried at 40 °C in a drying oven to obtain sericin solid for Fourier transform infrared (FTIR) spectroscopy. We chose storage durations of 1 day and 10 days, because the gelation of sericin does not complete at 1 day, but completes at 10 days. Hence, we chose these storage durations to examine the molecular conformation of sericin before and after complete gelation.

To fabricate sericin films, two different casting solvents (water and formic acid) were used [19]. In case of casting with water, the 1.0% (w/w) the aqueous solutions of sericin with different MWs were poured into Petri dishes and cast at 80 °C in the drying oven to obtain the sericin films. In case of casting with formic acid, the sericin solid powder was dissolved in 98% formic acid at 55 °C for 30 min to prepare 1.3 and 1.4% (w/w) sericin solutions. The formic acid solutions of sericin samples were poured into Petri dishes and dried in a fume hood at 25 °C for two to three days to obtain the sericin films.

To fabricate sericin sponges, the 2.5% (w/w) aqueous sericin solutions were frozen at −50 °C for 20 h and thawed for 4 h at room temperature [17]. This freezing-thawing process was repeated four times, and then the thawed sericin samples were frozen again for 20 h. The frozen sericin samples were freeze-dried in a freeze-dryer (PVTFD20R, Ilshin Biobase, Gyeonggi-Do, South Korea). Finally, sericin sponges with different MWs were fabricated. Physical cross-linking between sericin molecules occurs during the repeated freezing/thawing, leading to improvement in the mechanical properties of the resulting sponge. Fig. 1 shows the representative sericin film and sponge prepared in this study.

2.3. Measurement and characterization

The MW distribution of the sericin samples was determined by gel permeation chromatography (GPC) (ÄKTA Purifier, GE Healthcare, USA) using a Superdex 200 10/300 GL column (GE Healthcare, USA) as the solid phase. To prepare the samples, the lyophilized sericin samples were dissolved in a 4 M urea aqueous solution and 0.1% (w/v) solutions were filtered through a 0.2-he syringe filter before the GPC analysis. For each sample, a 100 r volume of the solution was injected and the mobile phase (4 M urea) of the 1.5-column volume was flown at a constant flow rate of 0.5 mL/min. The concentration of the protein was monitored by its UV absorbance at 280 nm. The MW was determined using a calibration curve obtained with the standard globular protein kits provided by GE Healthcare for calibrating the column [32,41,42].

The gelation behavior and gel-sol transition of the sericin sample were examined using a rheometer with 35-mm parallel plate geometry at 25 °C. An axial test was conducted to analyze the gelation behavior and the speed of compression was 0.2 mm/s. An oscillation temperature sweep test was conducted to determine the gel-sol transition. The temperature was controlled from 20 to 100 °C and the ramp rate was 20 °C/min. For the rheological tests, strain and frequency of 0.01% and 1 Hz, respectively, were used [16,17,19].

To examine the molecular conformation, the proportion of the molecular conformations, and the crystallinity index of the sericin with different MWs, FTIR spectroscopy (Nicolet 380, Thermo Fisher Scientific, USA) was performed in the attenuated total reflection method. The scan range was 4000 cm^{-1} to 650 cm^{-1} and the scan number and the resolution were 32 and 8 cm^{-1}, respectively.

The proportion of the molecular conformations was estimated by deconvoluting the amide I band (1600 to 1700 cm^{-1}) of sericin using the Fourier self-deconvolution fitting method in the Origin 8.0 software [37,43]. The crystallinity index was also calculated from the FTIR spectrum as the intensity ratio of the 1645 and 1616 cm^{-1} bands, using Eq. (1) [16,19].

$$\text{Crystallinity index}\ (\%) = \frac{A_{1616cm^{-1}}}{A_{1645cm^{-1}} + A_{1616cm^{-1}}} \times 100 \quad (1)$$

Table 1
Sample codes and preparation conditions of silk sericin with different MWs.

Sample code	First hot water treatment time (liquor ratio = 1:10, 1:25)	Second hot water treatment time
SS-10	10 min	–
SS-30	30 min	–
SS-30-30	30 min	30 min
SS-30-90	30 min	90 min