Characteristics of Silk Fiber with and without Sericin Component: A Comparison between Bombus mori and Philosamia ricini Silks

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Abstract: The study aimed to investigate and compare some characteristics of Bombus mori and Eri (Philosamia ricini) silks in different forms, with and without sericin. The protein contents were measured and find out the composition of the silk fibroin and sericin proteins by Lowry method. The secondary structure and thermal behavior of all kind of silk were determined by FT-IR and TA instrument, respectively. The B. mori composed of more amount of sericin content than that of Eri silk. FT-IR spectra indicated that the Eri silk was similar profile of silk with and without sericin, whereas B. mori silk showed dramatically differ. With sericin, B. mori composed of higher ratio random coil and α-helix structures than β-structure. With thermogravimetric analysis, both B. mori and Eri silk fibers without sericin showed higher stability than that silk fiber with sericin. This is due to the crystalline region of hydrophobic amino acid composed in the fibroin core protein. The differential scanning calorimetry thermogram of B. mori was differed from Eri silk fiber. It is a promising that characteristics of the silk were influenced by both silk components and silk varieties.

Key words: Silk, fibroin, sericin, FT-IR, thermal stability

INTRODUCTION

Silk is natural fibrous protein which was spun by Lepidoptera larvae such as silkworms, spiders, scorpions, mites and flies (Jin et al., 2002). Recently, silk has been focused as biotechnological and biomedical resources. These were due to their unique properties including nontoxicity, biocompatibility and biodegradability ( Foo and Kaplan, 2002).

Silkworm is a kind of insect which can be produced silk solution. The silk fibers were mostly spun by the family Bombycidae (domestic silk; Bombus mori) and Saturniidae (wild silk; Antheraea pernyi, Philosamia ricini, etc.) of the order Lepidoptera (Dash et al., 2007). Each silk fiber is composed of two sub-fibers. Each of which fibroin is the core. The two cores are coated and wrapped as together with sericin, the glue-like proteins (Altman et al., 2003). Silk Fibroin (SF) shows excellent both physical and chemical properties and has been used in various fields such as cosmetics, food additives and medical materials (Min et al., 2004; Taddei et al., 2006). In addition, SF can be prepared in various forms such as gel, powder, film, matrix or fiber depending on applications (Park et al., 2004).

Wide variations in composition are observed, although only a few types of amino acid residues are dominant in silk. Moreover, variability can be influenced by the dietary intake of the animal and the environmental conditions during the spinning process ( Shao and Vollrath, 2000). Generally, silk fibers compose of at least three types: β-sheet, random coil or α-helical structure (Craig and Riekel, 2002). The chemical compositions by weight are, in general, 75-83% of silk fibroin, 17-25% of sericin, 1.5% of wax and about 1-2% of others such as hydrocarbon. Silk fiber is normally stable up to 140°C and the thermal decomposition is greater than 150°C. It is known that the density of silk fiber with sericin is in the range of 1320-1400 g m⁻³, whereas silk fiber without sericin is 1300-1380 g m⁻³, respectively (Lee et al., 2005).

So far, the different characteristics between B. mori and P. ricini (Eri) silks are little information available, especially conformation structure and thermal properties. Moreover, comparison of the characteristic of both silks on silk fibers with and without sericin was rarely done. In this study, silk fibers of B. mori and P. ricini composed of sericin and sericin free were investigated and compared both secondary structure and thermal stability. The goal of the study is to explore the characteristic information of different silks.

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MATERIALS AND METHODS

Materials: The P. nichii (En) and B. mori silk cocoons were kindly supplied from Silk Innovation Center (SIC), Mahasarakham University, Thailand. The cocoons were kept in an air-dried room until use. Chemical reagents in analytical grade were used.

Sample preparation: Both B. mori and Eni cocoons were washed with distilled water to exclude those of impurity and dried in oven at 40°C. The sericin protein was extracted from the cocoons by immersing in distilled water and then autoclave at 121°C for 20 min. The solution was used as substrate for further determination. In case of pure fibroin, silk cocoons were weighed before degumming with 0.5% (w/v) NaHCO₃ solution at 95°C for 30 min and rinsed with distilled water. This step was performed for 2 times to obtain the silk fibroin. The cocoons were dried in oven at 40°C and calculated of weight lost to find the percentage ratio of sericin and silk fibroin scaffolds. The silk fibroin solution was also prepared to measure protein contents. Silk fibroin of B. mori was dissolved by using the tetraethylammonium system of CaCl₂-Ethanol-H₂O (1:2:8 by mole), whereas silk fibroin of Eni was dissolved by using 10M Ca(NO₃)₂ with magnetic stirred at 90-95°C for 2-3 h. The silk fibroin solution was then dialyzed using dialysis bag against distilled water for 3 days to exclude salts. The obtained solutions were used for measurement of the protein quantity.

Sericin and fibroin measurement: Protein determination was done as followed by Lowry procedure (Lowry et al., 1951).

Secondary structure analysis: Those of silk samples were analyzed for their secondary structure using FT-IR spectrometer (Perkin Elmer-Spectrum Gx, USA) in the spectral region of ~2000-500 cm⁻¹ at 4 cm⁻¹ spectral resolution and 32 scans.

Thermal behavior measurement: About 8-10 mg of individual silk samples was loaded in a platinum crucible. The thermogravimetric analysis (TGA) was then performed using TA instruments, SDT Q600 (Lukens's drive, New Castle, DE). The samples were non-isothermal heated from 50 to 1000°C at a heating rate of 10°C min⁻¹. The TGA was carried out in nitrogen with the flow rate of 100 mL min⁻¹. The TG and DSC data were recorded with TA instrument’s Q series explorer software. The analyses of the data were done using TA Instrument’s Universal Analyses 2000 software (version 3.3B).

RESULTS

Protein measurement: The protein content of sericin and fibroin is given in Table 1. It was found that silk cocoon composed of 31.06% sericin and 68.94% fibroin for B. mori, while Eni was 15.74% sericin and 84.26% fibroin. With Lowry method, the protein content of sericin was 0.2705 mg mL⁻¹ and fibroin was 0.3581 mg mL⁻¹ for B. mori whereas Eni composed of 0.0561 mg mL⁻¹ sericin and 0.0380 mg mL⁻¹ of fibroin content.

FT-IR spectra: B. mori silk fibers both with and without sericin were dramatically differed in absorption bands of those amide I, II, III and IV as shown in Fig. 1. The Eni silk with sericin showed the absorption bands at 1649 cm⁻¹ (amide I), 1559, 1523 cm⁻¹ (amide II), 1233 cm⁻¹ (amide III) and 964 cm⁻¹ (amide IV). Eni silk without sericin showed absorption bands almost similar to silk with sericin, especially at amide I, II and III. However, the absorption band of amide IV slightly shifted. Contrast, B. mori silk fiber showed dramatically different absorption bands compared between silk with and without sericin. Generally, the absorption bands of B. mori silk with sericin appeared at 1649 cm⁻¹ (amide I), 1506, 1523 cm⁻¹ (amide II), 1234 cm⁻¹ (amide III) and 924 cm⁻¹ (amide IV). The B. mori without sericin showed intense absorption bands at 1643 cm⁻¹ (amide I), 1509 cm⁻¹ (amide II) and 1127 cm⁻¹ (amide III). It was also found that the absorption band of amide IV was gone. Those of the absorption bands of B. mori silk fiber shifted to lower wave length number after removing of sericin.

Table 1: Percentage and contents of sericin and fibroin proteins

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<th>B. mori</th>
<th>Eni</th>
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<tr>
<td>Percentages (%)</td>
<td>31.60</td>
<td>15.74</td>
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<tr>
<td>Protein content (mg mL⁻¹)</td>
<td>0.2705</td>
<td>0.0561</td>
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Fig. 1: FT-IR spectra of the different forms of silk fiber; (A) B. mori with sericin, (B) B. mori without sericin, (C) Eni with sericin and (D) Eni without sericin.
Fig 2: Thermogravimetric curves of the silk fibers in different components

Fig 3: DTG curves of the silk fibers in different components

Fig 4: DSC thermograms of the silk fibers in different components

Thermal decomposition analysis: From the thermogravimetric (TG) curves in Fig. 2, the initial weight loss at below 100°C was due to the water evaporation (Kweon et al., 2001). At temperature over 200°C, the weight loss was occurred again. Generally, B. mori silk has decomposed weight faster than that of Eri. B. mori silk showed different TG curves between the silk fibers with and without sericin. Contrast result was observed for Eri silk since it has almost decomposed temperature at the same point of both silk fibers. However, the silk did not completely decompose even at 1000°C. The result found that Eri silk underwent at least three thermal decomposition stages, which are 200-300°C, 300-350°C and 350-400°C. On the other hand, B. mori silk has single step of thermal decomposition at 350 and 360°C for silk fiber with and without sericin, respectively. The detail of those decomposition peaks were clearly evidenced by differential thermogravimetric (DTG) curves (Fig. 3). The maximum decomposition temperatures of Eri silk with sericin were 230, 330, 385°C, while Eri silk without sericin were 235, 325 and 390°C. The decomposition temperatures of both kind silks were higher than that of B. mori silks since the maximum thermal decomposition of them took place only single step at 325 and 340°C for silk with and without sericin, respectively.

Differential scanning calorimetry: With DSC thermograms, the peak at below 100°C distributions to the moisture loss of the SF appeared in all of samples. The Eri silk showed multiple endothermic peaks at approximately 240, 320, 335, 450°C and 240, 340, 390, 460°C for silk with and without sericin. On the other hand, the B. mori silk occur strong single step at 329 and 334°C for silk with and without, respectively (Fig. 4).

DISCUSSION

The silk fibers were investigated and compared. The results found that protein contents, secondary structure and thermal properties of B. mori and Eri silks were dramatically differed. In addition, differed characteristics between the silk fibers with and without sericin were also observed and reported. It is well known that silk fiber composed at least two main proteins, sericin and fibroin (Jin et al., 2002). Both sericin and fibroin were investigated to find the percentage and protein contents. The results found that sericin of B. mori composed in higher (31.6%) level that Eri (15.74%) silk. Moreover, protein concentration of B. mori (0.2705 mg mL⁻¹) was also higher than that of Eri (0.0561 mg mL⁻¹) silk. It is relate to other reports that the sericin protein of domesticated silk (B. mori) was higher content than wild silk. The different compositions might be affected to other properties such as secondary structure and thermal behavior. The secondary structures of protein are indicated by the amide groups of the silk composition (Kweon et al., 2000, Hino et al., 2003). The FT-IR results
indicated that the main structure of silk with and without sericin of B. mori showed different profiles. With sericin, silk is existed of random coil and α-helix structures. Without sericin, the wave length was dramatically shifted to lower indicating the β-sheet structure was formed (Tao et al., 2007). On the other hand, Eri silk did not change the spectra. The results showed similar with previous reported notes that the sericin has influenced on the strength and luster of silk fiber. The result suggested that low sericin content in the Eri silk is main factor. Thermal properties of domesticated silk, B. mori took place in a single step; contrast from wild silk, P. ricini which underwent at least two steps (Kweon et al., 2000). This might be said that structure and amino acid between B. mori and Eri silk are different. Furthermore, TG and DTG results obviously showed that B. mori silk with and without sericin have different trends on thermal decomposition. Both B. mori and Eri silks without sericin showed higher thermal stability than that silk with sericin. This point might differ from the comment of previous finds that sericin component should enhance the strength of the silk fiber. However, with the results of thermal behavior that sericin free has high thermal stability. This suggested that the rearrangement of the amino acid components after excluding sericin was influenced on the silk properties. This illustrated that the process of sericin removing might be enhanced the crystalline formation by changing the structure from random coil to β-sheet structure since the β-sheet structure could be formed by those of the fiber reinforcement and matrix, fiber content, fiber length, fiber orientation, processing method and condition (Sofia et al., 2001).

CONCLUSION

The different details of protein contents, secondary structure and thermal properties of B. mori and Eri silk both with and without sericin were reported in this study. Sericin, one main component of silk was compared between domesticated and wild silk. It was found that B. mori (domesticated silk) has higher sericin content than Eri (P. ricini, wild silk). This different composition was affected to other properties including secondary structure and thermal stability. The results showed that the B. mori silk with and without was dramatically differed in FT-IR spectra, but did not for Eri silk. Those of TG, DTG and DSC indicated that the thermal behavior was differed between silk with and without sericin content. Silk without sericin showed higher stability than another one. In conclusion, different characteristics could be affected by silk composition as well as silk varieties.

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REFERENCES


