http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (11): 872-876, 2009
ISSN 1028-8880

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

[1]S. Prasong, [1]S. Yaowalak and [2]S. Wilaiwan
[1]Center of Excellence for Innovation in Chemistry, Department of Chemistry, Faculty of Science, Mahasarakham University, 44150, Thailand
[2]The Central Instrument, Faculty of Science, Mahasarakham University, 44150, Thailand

Abstract: The study aimed to investigate and compare some characteristics of *Bombyx 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 differed. 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; *Bombyx 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 coin 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.

Corresponding Author: Srihanam Prasong, Center of Excellence for Innovation in Chemistry, Department of Chemistry, Faculty of Science, Mahasarakham University, 44150, Thailand Tel: +66-43-754246 Fax: +66-43-754246

MATERIALS AND METHODS

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

Sample preparation: Both *B. mori* and Eri 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) $NaHCO_3$ 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 tertiary system of $CaCl_2$-Ethanol-H_2O (1:2:8 by mole), whereas silk fibroin of Eri was dissolved by using 10M $Ca(NO_3)_2$ 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 (Luken'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 Analysis 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 31.60% sericin and 68.40% fibroin for *B. mori*, while Eri 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 Eri composed of 0.0561 mg mL^{-1} sericin and 0.0830 mg mL^{-1} of fibroin content.

FT-IR spectra: *B. mori* silk fibers both with and without of sericin were dramatically differed in absorption bands of those amide I, II, III and IV as shown in Fig. 1. The Eri 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). Eri 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), 1560, 1523 cm^{-1} (amide II), 1234 cm^{-1} (amide III) and 1024 (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 of wave length number after removing of seicin.

Table 1: Percentage and contents of sericin and fibron proteins

	B. mori		Eri	
List	Sericin	Fibron	Sericin	Fibron
Percentage (%)	31.60	68.40	15.74	84.26
Protein contents (mg mL^{-1})	0.2705	0.3581	0.0561	0.0830

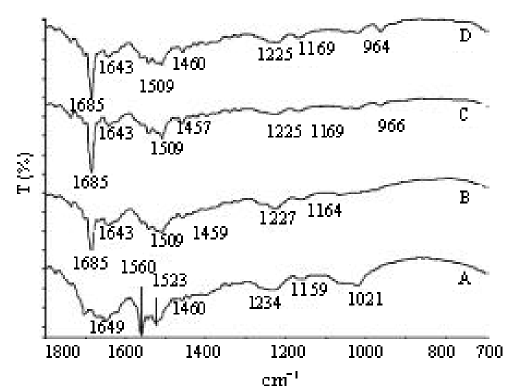

Fig. 1: FT-IR spectra of the different forms of silk fiber; (A) *B. mori* with sericin, (B) *B. mori* without sericin, (C) Eri with sericin and (D) Eri without sericin

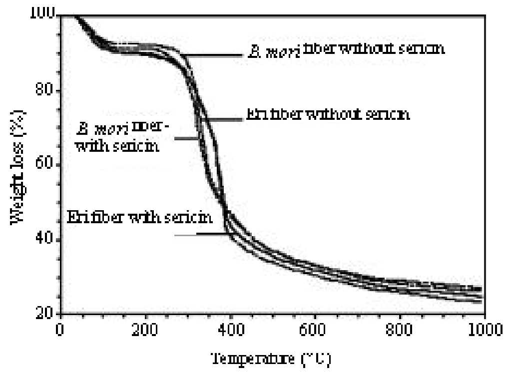

Fig. 2: Thermogravimetric curves of the silk fibers in different components

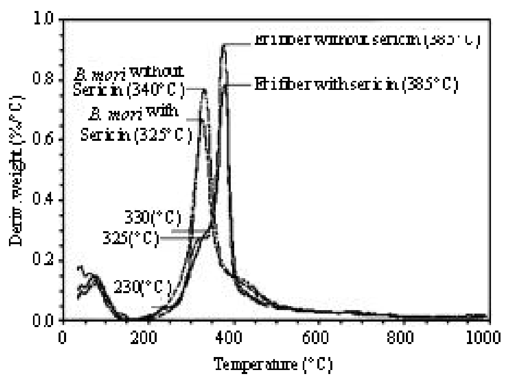

Fig. 3: DTG curves of the silk fibers in different components

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 of 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

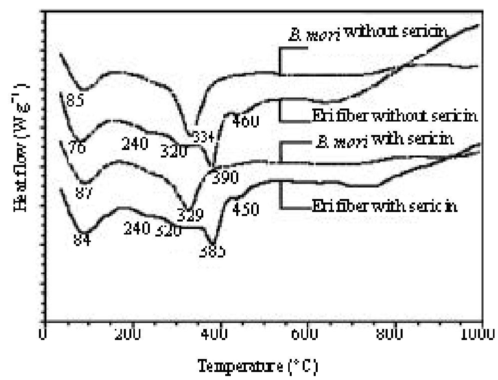

Fig. 4: DSC thermograms of the silk fibers in different components

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, 385, 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.

ACKNOWLEDGMENTS

We appreciate to thank Division of Support and Development, Mahasarakham University for financial support. We also thank the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education, Thailand to support this study.

REFERENCES

Altman, G.H., F. Diaz, C. Jakuba, T. Calabro and R.L. Horan *et al*., 2003. Silk-based biomaterials. Biomaterials, 24: 401-416.

Craig, C.L. and C. Riekel, 2002. Comparative architecture of silks, fibrous proteins and their encoding genes in insects and spiders. Comp. Biochem. Physiol. B: Biochem. Mol. Biol., 133: 493-507.

Dash, R., S.K. Ghosh, D.L. Kaplan and S.C. Kundu, 2007. Purification and biochemical characterization of a 70 kDa sericin from tropical tasar silkworm, *Antheraea mylitta*. Comp. Biochem. Physiol. Part B, 147: 129-134.

Foo, C.W.P. and D.L. Kaplan, 2002. Genetic engineering of fibrous proteins: Spider Adv. Drug Deliver Rev., 54: 1131-1143.

Hino, T., M. Tanimoto and S. Shimabayashi, 2003. Change in secondary structure of silk fibroin during preparation of its microspheres by spray-drying and exposure to humid atmosphere. J. Coll. Interf. Sci., 266: 68-73.

Jin, H.J., J. Park, R. Valluzzi, P. Cebe and D.L. Kaplan, 2002. Biomaterial films of Bombyx mori silk fibroin with poly (ethylene oxide). Biomacromolecules, 3: 1233-1239.

Kweon, H.Y., I.C. Um and Y.H. Park, 2000. Thermal behavior of regenerated *Antheraea pernyi* silk fibroin film treated with aqueous methanol. Polymer, 41: 7361-7367.

Kweon, H.Y., I.C. Um and Y.H. Park, 2001. Structural and thermal characteristics of *Antheraea pernyi* silk fibroin-chitosan blend film. Polymer, 42: 6651-6656.

Lee, S.M., D. Cho, W.H. Park, S.G. Lee, S.O. Han and L.T. Drzal, 2005. Novel silk/poly (Butylene Succinate) biocomposites: The effect of short fibre content on their mechanical and thermal properties. Comp. Sci. Tech., 65: 647-657.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

Min, B.M., L. Jeong, Y.S. Nam, J.M. Kim, J.Y. Kim and W.H. Park, 2004. Formation of silk fibroin matrices with different texture and its cellular response to normal human keratinocytes. Int. J. Biol. Macromol., 34: 281-288.

Park, W.H., L. Jeong, D.I. Yoo and S. Hudson, 2004. Effect of chitosan on morphology and conformation of electrospun silk fibroin nanofibers. Polymer, 45: 7151-7157.

Shao, Z. and F. Vollrath, 2000. Surprising strength of silkworm silk. Nature, 418: 741-741.

Sofia, S., M.B. McCarthy, G. Gronowicz and D.L. Kaplan, 2001. Functionalized silk-based biomaterials for bone formation. J. Biomed. Mater. Res., 54: 139-148.

Taddei, P., T. Arai, A. Boschi, P. Monti, M. Tsukada and G. Freddi, 2006. *In vitro* study of the proteolytic degradation of *Antheraea pernyi* silk fibroin. Biomacromolecules, 7: 259-267.

Tao, W., M. Li and C. Zhao, 2007. Structure and properties of regenerated *Antheraea pernyi* silk fibroin in aqueous solution. Int. J. Biol. Macromol., 40: 472-478.