International Journal of Biological Chemistry

ISSN 1819-155X

Academic Journals Inc.

www.academicjournals.com

International Journal of Biological Chemistry 3 (2): 78-83, 2009
ISSN 1819-155X

Effect of Methyl Alcohol on Conformational Structure and Thermal Behavior of Eri (*Philosamia ricini*) Silk Fibroin Film

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Abstract: This study aims to prepare silk fibroin film of Eri (*Philosamia ricini*) and investigate their conformational structure and thermal properties after treating with methyl alcohol. The Eri silk fibroin solution was obtained by dissolving the Eri cocoons with 9 M $Ca(NO_3)_2$. The silk fibroin hydrolysate was dialyzed to remove the concentrated salt against distilled water. It was then concentrated to give 2% silk fibroin by weight. Ten milliliter diluted silk fibroin to 0.5% weight per polystyrene plate was used to cast film at room temperature for 2 days. The obtained films were then treated with 80% methyl alcohol with different times. They were subjected to investigate using Fourier transform infrared spectroscopy (FTIR) and thermogravimetric analyzer for conformational and thermal studies, respectively. The FT-IR spectra showed that Eri silk films were composed of both α-helix and β-sheet structures before exposure to alcohol and changed from low content of β-sheet structure to higher ratio when immersed in alcohol. In addition, the β-sheet structure gradually increased according to the increase of treating time used. The relative data were obtained from thermal investigation since the decomposition temperatures of Eri silk fibroin films were increased as follow by the increase of methyl alcohol treating time. It is promising that methyl alcohol can be affected to change both conformation and thermal behavior of the Eri silk fibroin film.

Key words: Silk fibroin, conformational structure, thermal properties, methyl alcohol

INTRODUCTION

Silk is a natural fiber spun by different types of insects, especially silkworm (Jin *et al*., 2002). Generally, silk was divided into mulberry or domesticated silk (as well known; *Bombyx mori*) and non-mulberry or wild silk. Silk polymer is growing interests for textile and biomaterial in recent years. It has also been promoted various studies on their structure and properties (Kweon *et al*., 2000). The advantages of silk properties including unique strength, biodegradability, water and oxygen vapor ability as well as biocompatibility are interested to study (Altman *et al*., 2003; Min *et al*., 2004). In addition, silk can modulate in various forms to match of working requirements such as film, powder, gel, membrane and so on (Altman *et al*., 2003). Among the natural polymers, wild silk has been interested in study and applications (Kweon *et al*., 2001). It is a remarkable type of the material for supplying or supplements the utilization of other polymers.

Eri silk (*Philosamia ricini*) is a wild silk that has been cultured routinely in many countries, especially in China, India, Japan, Korea and Thailand (Mishra *et al*., 2003). All most of silk yarn production of Eri were used in textile and little of information for specific character both chemical and physical properties. The study on those of Eri properties is very interested to provide the general data of this silk, especially silk fibroin film. The important points for application silk are their structural and physical features.

Generally, silk fibroin existed of three crystalline forms, i.e., silk I, II and III. The silk I is the metastable structure of fibroin and easily transferred into silk II; β-sheet conformation with many ways such as stretching (Chang *et al*., 2005), thermal treatment (Freddi *et al*., 1997) or immersion in organic solvents (Tasukada *et al*., 1995).

Study on the conformational structure and thermal properties of Eri (*P. ricini*) after treating with methyl alcohol were rarely reported. Therefore, Eri silk fibroin films were prepared. The obtained films were then determined to detail of their structure and thermal properties.

MATERIALS AND METHODS

This study was constructed for 4 months from December 1, 2008 to March 30, 2009. All of Eri film preparation was performed at Room 406 and thermogravimetric analysis was determined at Rooms 404, SC 1 Building, Department of Chemistry, Faculty of Science. Examination of conformational structure by FT-IR was done at Central Instrument, Faculty of Science, Mahasarakham University, Thailand.

Materials

The Eri (*P. ricini*) silk cocoons were kindly supplied from Silk Innovation Center (SIC) Mahasarakham University, Thailand. The cocoons were strips and then degummed twice using 0.5% Na_2CO_3 (w/v) and thoroughly rinsed 2 times in warm distilled water. They were then dried at room temperature before dissolving.

Dissolution of Silk

The Eri silk was dissolved with 9M $Ca(NO_3)_2$ solution. The method was applied from Tao *et al*. (2007). Briefly, dried Eri SF and the solution were mixed at the rate of 1 g fibroin to 10 mL of 9 M $Ca(NO_3)_2$ solution. The solution was firstly warmed to about 90°C, then gradually added SF into the solution and stirred with controlled temperature at 100-105°C until silk already dissolved which taken approximately 45 min. The SF hydrolysate was filtrated and then dialyzed in cellulose tube against distilled water for 3 days at room temperature and concentrated into 2% by weight.

Preparation of Silk Fibroin Films

The Eri silk solution with 0.5% (w/w) was stirred and then cast on the 5 cm diameters polystyrene plates each with 10 mL. The plates were left air dried at room temperature for 3 days. Finally, the films with a thickness about 30-45 μm were obtained.

Methyl Alcohol Treatment

The Eri films were immersed in 80% aqueous methyl alcohol in different times that are 30, 60 and 90 min. After finish in each time, they were left in air-dried and transferred into vacuum oven to make sure the films were completely dried. They were stored in desiccator prior to use.

Silk Fibroin Film Investigation

The Eri films were analyzed for their structure with FT-IR (Perkin Elmer-Spectrum Gx, USA) in the spectral range of ~4000-740 cm^{-1} at 4 cm^{-1} spectral resolution and 32 scans. FT-IR is a sensitive tool used to measure the absorption bands which represented the silk structure. A TA-Instrument TG SDT Q600 thermogravimetric analyzer was used to determine the thermal behavior of the films. The analysis condition was 50-1,000°C for heating sample at 20°C min^{-1} rates under nitrogen atmosphere.

RESULTS

FT-IR Spectra

Functional groups of the SF component and their intermolecular interactions are determined from FT-IR spectra as shown in Fig. 1. For amide I band, all of samples including control (no treat) showed the strong absorption at 1655 cm^{-1}, which is the characteristic absorption band of α-helix structure. However, many absorption bands were also observed when the SF film treated for 90 min. On the other hand, amide III (~1235 cm^{-1}) was similar profiles most of samples. The obvious difference of the absorption bands were indicated by amide II. The band occurred at 1558 cm^{-1} in the control, distributed to α-helix structure. After treating with methyl alcohol for 30 min, the amide II shifted to 1553 cm^{-1}. This point is considered to α-helix band. However, the structure was changed when treated the SF film for 60 min. From the FT-IR spectrum, the absorption band occurs at 1525 cm^{-1} (amide II, β-sheet). In addition, the higher content of β-sheet were achieved by treating for 90 min in methyl alcohol (1522 cm^{-1}) (Tao *et al*., 2007; Kweon *et al*., 2001).

Thermal Behavior

The thermal behavior of the SF films treated with methyl alcohol in different times was studied from Thermogravimetric (TG) curves as shown in Fig. 2. The results showed that the initial weight loss at around 100°C was the evaporation of water. All of SF films showed similar of their even on different treatment times. To clearly evidence of the thermal behavior of the SF films, the data were obtained from the Differential thermogravimetric (DTG) curves (Fig. 3). Those of the maximum tolerances to the temperature of the Eri SF films were at about 320°C with shoulder at about 362°C.

Differential Scanning Calorimetry

The transition stages of the SF films after treating with methyl alcohol were observed from Differential Scanning Calorimeter (DSC) curves (Fig. 4). Those of regenerated SF appeared sharp endothermic peaks at about 300°C. Moreover, the endothermic at about 380°C almost disappeared when the film was treated with methyl alcohol for over 60 min, which can be explained by the FT-IR result. Exothermic peaks at about 510°C were also observed when treated the Eri SF films for over 60 min.

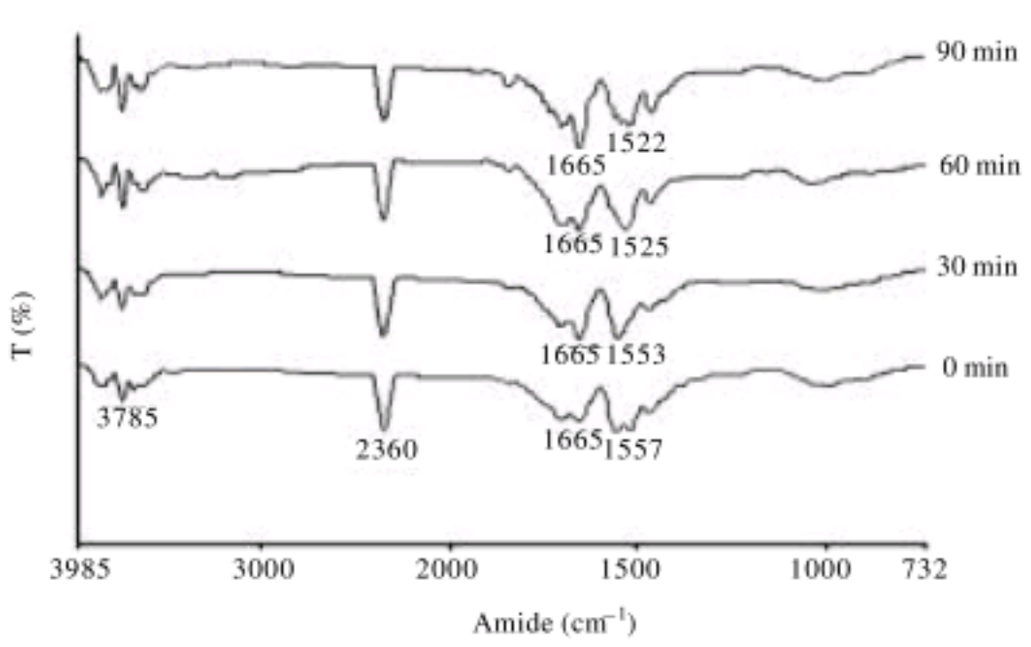

Fig. 1: FT-IR spectra of SF forms treated with methyl alcohol in different times

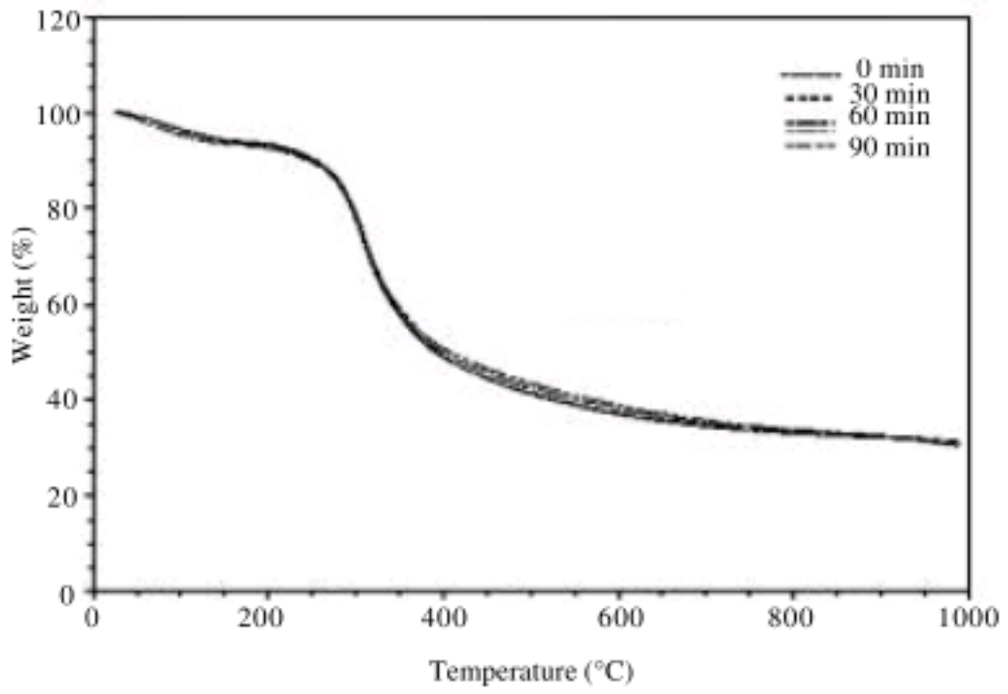

Fig. 2: Thermogravimetric curves SF forms treated with methyl alcohol in different times

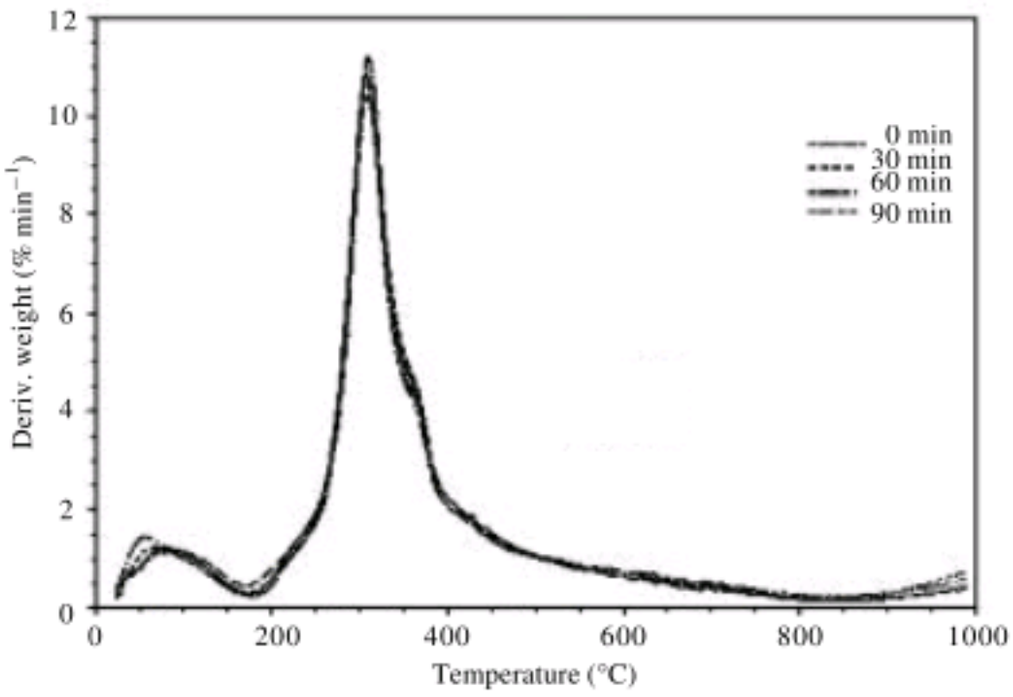

Fig. 3: DTG curves of SF forms treated with methyl alcohol in different times

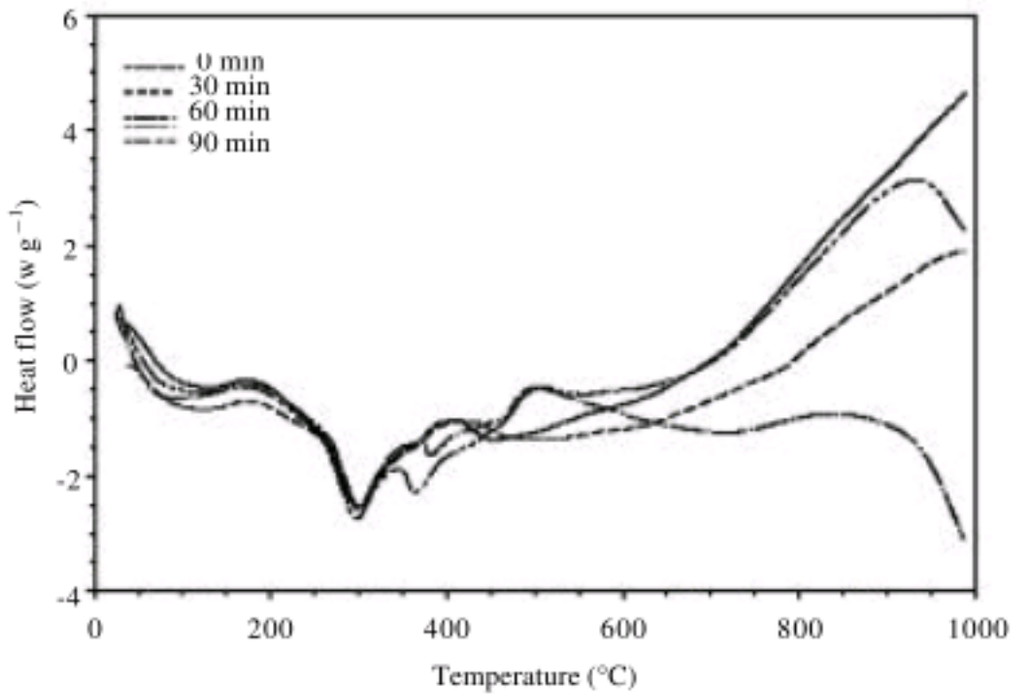

Fig. 4: DSC curves of SF forms treated with methyl alcohol in different times

DISCUSSION

The Eri SF could be dissolved by using the concentrate salt of 9 M $Ca(NO_3)_2$. The SF solution was then cast on the plates to obtain the SF film. Those of the SF film are transparency and easy to broke. To study the secondary and thermal properties, they were treated with methyl alcohol since this solvent has been reported to enhance the strength of the material. This study achieved to prepare SF film to use as subject to immerse in the solvent.

Secondary Structure

Generally, analysis of secondary structures of protein are indicated by the absorption bands; amide I, amide II and amide III from IR spectra (Kweon *et al*., 2000; Hino *et al*., 2003). This may be thought that the intra-molecule inside the film formed bond as well as rearrangement of molecules closed together. This characteristic can enhance the strength of the film by formation of silk crystalline. In contrast, the film is also brittle and more fragile than the film that composed more α-helix structure. The FT-IR spectra revealed that the increase of β-sheet was affected by the treating time used. However, those of the absorption bands were differed from other wild silk with report previously (Kweon *et al*., 2000). This is a promising that conformational structures of the SF were mainly differed by different types of silk.

Decomposition Profiles

Both weight loss and DTG curves did not significantly change even the treatment time by methyl alcohol was increased. The results indicated that Eri SF films attributed to the disintegration of intermolecular interaction and some break of their structure (Kweon *et al*., 2001). It is thought that immersing in methyl alcohol did not affect on the thermal behavior of Eri SF film. The results were similar with the previously report which was observed in *A. pernyi* (Kweon *et al*., 2000).

Endo/Exo Transitions

It is well known that this point is according to the fibroin compositional molecules with unoriented β-sheet conformations decomposed (Kweon *et al*., 2000). This indicates that the random coil structure was changed into β-sheet completely. The results illustrated that the β-sheet structure of the regenerated films can be increased as the treatment time with methyl alcohol increased. Similar in profile was also reported by *A. pernyi* SF (Kweon *et al*., 2000). However, both endo/exo transition points were significantly differed when compared with other wild SF, *A. pernyi*.

CONCLUSION

Both conformational structure and thermal properties of the Eri SF films are required to apply this silk in many fields. It is confirmed that 80% methyl alcohol treatment can be enhanced the conformational structure change from random coil to β-sheet structure which indicated by FT-IR spectra. In addition, the β-sheet formation was increased by extending the exposure times in methyl alcohol. With TG and DTG curves, the treatment time seem to little affect on the thermal behavior of the Eri SF film. However, DSC thermograms have an evidenced that exo/endo transition peaks were great dependent by the methyl alcohol treatment time.

ACKNOWLEDGMENTS

The author would like to thank Silk Innovation Center, Mahasarakham University for kindly supplied of silk cocoons, Central Instrument Unit and Chemistry Department, Faculty of Science,

Mahasarakham University for conveniences analysis facilities. In addition, gratefully acknowledge is also to the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education, Thailand for financial support.

REFERENCES

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

Chang, J.C., M.J. Fletcher, G.M. Gurr, D.S. Kent and R.G. Gilbert, 2005. A new silk: Mechanical, compositional and morphological characterization of leafhopper (*Kahaono montana*). Polymer, 46: 7909-7917.

Freddi, G., P. Monti, M. Nagura, Y. Gotoh and M. Tsukada, 1997. Structure and molecular conformation of tussah silk fibroin films: Effect of heat treatment. J. Polym. Sci. Polym. Phys. Edu., 35: 841-847.

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., S.V. Fridrikh, G.C. Rutledge and D.L. Kaplan, 2002. Electrospinning *Bombyx mori* silk 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.

Min, B.M., G. Lee, S.H. Kim, Y.S. Nam, Y.S. Lee and W.H. Park, 2004. Electrospinning of silk fibroin nanofibers and its effect on the adhesion and spreading of normal human keratinocytes and fibroblasts *in vitro*. Biomaterial, 25: 1289-1297.

Mishra, N., N.C. Hazarika, K. Narain and J. Mahanta, 2003. Nutritive value of non-mulberry and mulberry silkworm pupae and consumption pattern in Assam, India. Nutr. Res., 23: 1303-1311.

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.

Tasukada, M., G. Freddi, P. Monti, A. Bertoluzza and N. Kasai, 1995. Structure and molecular conformation of tussah silk fibroin films: Effect of methanol. J. Polym. Sci. Polym. Phys. Edu., 33: 1995-2001.