IJIE Int. J. Indust. Entomol. 30(1) 1-5 (2015)
ISSN 1598-3579, http://dx.doi.org/10.7852/ijie.2015.30.1.1

Monitoring of phase separation between silk fibroin and sericin using various dye system

Hyo Won Kwak[1] and Ki Hoon Lee[1,2,3]*

[1]*Department of Biosystems & Biomaterials Science and Engineering, Seoul National University, Seoul 151-921, Korea*
[2]*Center for Food and Bioconvergence, Seoul National University, Seoul 151-921, Korea*
[3]*Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea*

Abstract

Understanding the interactions between fibroin and sericin is crucial in solving the mechanism of silk spinning. In this study, various commercially available dyes were used to monitor the interface between fibroin and sericin during the gelation of fibroin. The phase separation between fibroin and sericin could be observed by the addition of azo dyes over a certain molecular weight. Furthermore, the addition of the dyes to the sericin layer showed vivid phase separation over addition to the fibroin layer.

Int. J. Indust. Entomol. 30(1), 1-5 (2015)

Received : 29 Dec 2014
Revised : 24 Mar 2015
Accepted : 25 Mar 2015

Keywords:
Silk,
fibroin,
sericin,
dye,
interface

Introduction

Silk is one of the oldest fibers known to man and has been widely used in the textile industry. Recently, a variety of advantages for silk derived products have emerged. Silk protein has excellent mechanical properties, biocompatibility and biodegradability (Kundu *et al*., 2013). Due to these properties, various studies have been conducted on silk for biomedical and industrial applications (Okabayashi *et al*., 2009; Wang *et al*., 2008, 2010).

The silk fiber spinning process has the advantage of using water as a solvent at room temperature and atmospheric pressure (Jin and Kaplan, 2003). Silk fiber derived from the cocoon of *Bombyx mori* is composed of two proteins named fibroin and sericin. Sericin completely covers two strands of fibroin to form silk fibers. So far, the role of sericin during spinning process of silk fiber is unclear. Lee et.al proposed that sericin has a retardation effect on the crystallization of fibroin in the phase separation of the two proteins (Lee, 2004). In order to fully characterize the silk spinning process, the interactions between fibroin and sericin must be identified. However, it is difficult to observe the interface between the two proteins because both fibroin and sericin have transparent property in aqueous solutions.

A variety of dyes and dyeing processes have been developed for the use of silk in the textile industry (Tsukada *et al*., 1996). Recently, a large amount of research has focused on developing silk fibers with a desired color by introducing food dye to the silkworm breeding process (Tansil *et al*., 2011, 2012). Production of colored silk using this process is eco-friendly and efficient compared to conventional dyeing processes.

***Corresponding author.**
Ki Hoon Lee
Department of Biosystems & Biomaterials Science and Engineering, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-921, Korea
Tel: +82-2-880-4625 / FAX: +82-2-873-2285
E-mail: prolee@snu.ac.kr

However, a disadvantage of this type of dyeing process is that the concentration of dye transmitted to fibroin and sericin can be different depending on the dye. When a feeding process was employed using artificial feed laced with direct acid 80, almost all of the dye was transmitted to the fibroin layer. However, in the case of acid orange 80, most of the dye was transmitted to sericin layer (Nisal *et al*., 2014).

In this study, we prepared aqueous fibroin and sericin solutions laced with various dyes. We then observed the gelling behavior of fibroin that had been phase separated from sericin. Finally, an optimal dyeing system was determined for monitoring the interface of fibroin and sericin.

Materials and Methods

Materials

Bombyx mori silkworm cocoons were kindly provided by National Academy of Agricultural Science. All chemicals were purchased from Sigma-Aldrich (USA).

Extraction of fibroin

The silk cocoons were degummed twice with 0.2 % (w/v) sodium carbonate and 0.3 % (w/v) Marseille soap solution at 100°C for 30 min, and then rinsed with distilled water to remove any sericin. Following this, the fibroin was dried in an oven at 50°C.

Extraction and preparation of sericin aqueous solution

Sericin was extracted by boiling 20 g of *Bombyx mori* cocoons in 500 mL of distilled water using an autoclave for 1 h at 120 °C. The extractant was then filtered with a nonwoven filter (Miracloth, typical pore size: 22 ~ 25 μm, EMD Millipore, USA) in order to remove the remaining cocoons. After the filtering process, a 1 % (w/v) sericin solution was obtained.

Preparation of fibroin and sericin solutions with dyes

The previously extracted fibroin was dissolved (10%, w/v) in a 9.3 M LiBr solution at 50°C for 4 h. The solution was then dialyzed in distilled water with a cellulose membrane (molecular weight cut off = 6000 – 8000, Spectrum Laboratories, Inc.) for two days. The final fibroin concentration was adjusted 5 % (w/v) with distilled water. To prepare the dyed aqueous fibroin and sericin solutions, 100 ppm of each dye was added to each solution.

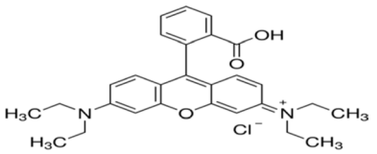
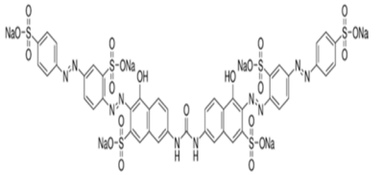
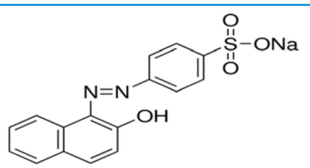
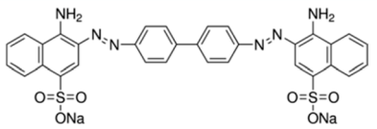
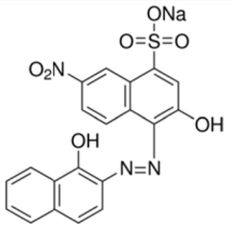
Monitoring the gelation behavior of fibroin

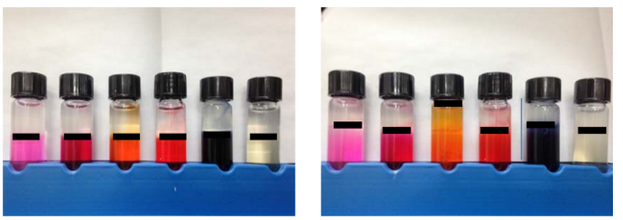
The gelation experiments of fibroin were carried out as follows. First, 3 mL of aqueous fibroin was added to a 5 mL vial using a pipette. Then, 1.5 mL of aqueous sericin was added to the above fibroin solution. In order to maintain the interface between the two solutions, sericin was added carefully using a syringe pump (KD Scientific, USA). The experiments were carried out in a situation supporting a dye in either the fibroin or sericin solutions.

Results and Discussion

During the silk fiber spinning process, sericin envelopes two strands of fibroin. However, the role of sericin in covering fibroin is still unclear. Oh *et al*. reported that during the gelation of fibroin under phase separated conditions with sericin, sericin retards the gelation time of fibroin. Additionally, the interface between fibroin and sericin was maintained until gelation (Oh *et al*., 2009). However, because both fibroin and sericin are transparent in aqueous solutions prior to gelation, monitoring the interface between the two solutions is difficult. Table 1 shows the name, molecular weight, and chemical structures of the five dyes used in the experiments. All of the compounds are azo dyes except for cationic rhodamine B. When staining or labeling materials with fluorescence compounds or dyes, it is very important that there is no interaction between the target material and the dyes (Joosen *et al*., 2014). We found that there was no change in the gelation time of the fibroin and sericin solutions with the addition of the dyes (data not shown). We performed phase separated gelation experiments using aqueous fibroin solutions (5%, w/v) with a dye concentration of 100 ppm and sericin solutions (1 %, w/v). Fig. 1 shows the phase separation between the fibroin and sericin solutions before and after gelation. As shown in Fig. 1, before gelation, all of the fibroin

Table 1. Molecular weight and chemical structure of dyes using this study

Dye	Molecular weight	Chemical structure
Rhodamine B	479.01	
Direct red 80	1373.7	
Orange II sodium salt	350.32	
Congo red	696.66	
Mordant Black 11	461.38	



(a) (b)

Fig. 1. Photograph of phase separation between fibroin and sericin solutions when dyed fibroin solutions were used. (a) before gelation, (b) after gelation

solutions with various dyes maintained phase separation with the sericin solutions. Following the gelation of fibroin, it was confirmed that all of the color separation was maintained except for orange II sodium salt. Fig. 2 shows the relative height of the dyed sericin solution after gelation compared to its initial height. The relative height increase for orange II sodium salt was 300 %,

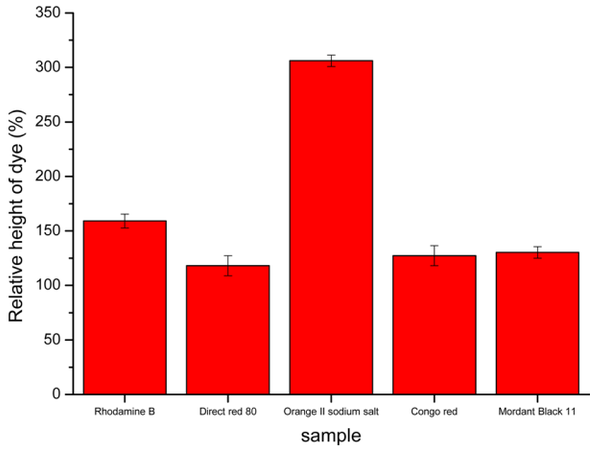
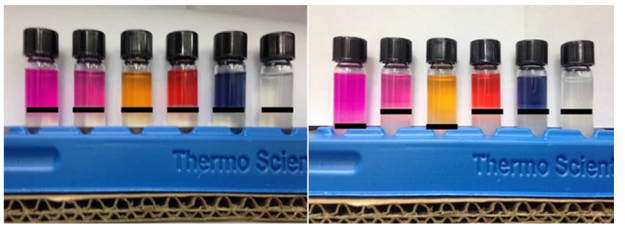


Fig. 2. Relative height of dyed solution compared to the height of the initial sericin solution.

suggesting that the dye diffused well into the sericin layer. This phenomenon is believed to be due to the molecular weight of dye. The molecular weight of orange II sodium salt is 350.32 g mol^{-1}, which is the lowest of the five dyes. Generally, the dyeing rate is proportional to the molecular weight of the dye. Because of the low molecular weight of orange II sodium salt, it could easily diffuse into the sericin layer. Comparing the remaining dyes, the relative height value for rhodamine B (159.1 %) was slightly higher than that of direct red 80 (118.2 %), Congo red (127.3 %) and mordant black 11 (130.3 %). This could be explained by the different dyeing mechanism of rhodamine B compared with the other dyes. Rhodamine B is a basic dye with a cationic group (Bhatnagar and Jain, 2005). This cationic group is attracted to and retained by the anionic sites of the fibers. The pH of fibroin and sericin solutions in the gelation experiments was 6.5 and 7.5, respectively which were higher than the isoelectric point of the two proteins. It means that aqueous fibroin and sericin solutions have negative net proton charge. This suggests that the abundance of negatively charged groups in fibroin and sericin could be easily attracted to rhodamine B. Therefore, the maintenance of phase separation between the two proteins with of the addition of rhodamine B is difficult.

To efficiently observe the interface between fibroin and sericin, we performed similar gelation experiments using dyed sericin aqueous solutions. The phase separation between the fibroin and sericin solutions before and after gelation and the relative height of the dyed solution after gelation compared to its initial height are shown in Figs. 3 and 4. Similar to the above experiment, when using direct red 80, Congo red and



(a) (b)

Fig. 3. Photograph of phase separation between fibroin and sericin solutions when a dyed sericin solution was used. (a) before gelation, (b) after gelation

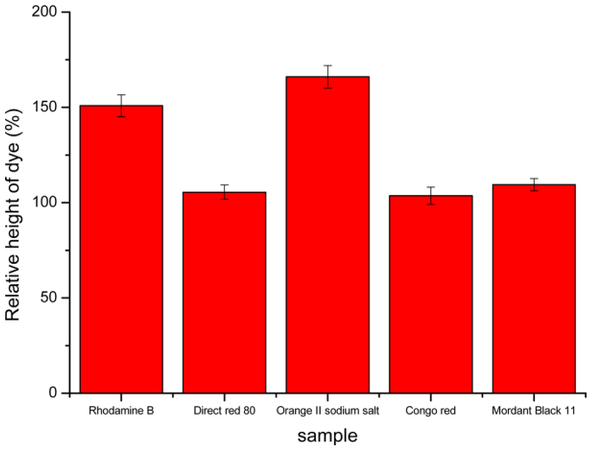


Fig. 4. Relative height of the dyed solution compared to height of the initial sericin solution.

mordant black 11 dye, phase separation between sericin and fibroin solutions was well maintained. In the case of rhodamine B and orange II sodium salt, diffusion of dye from the sericin to the fibroin layer was observed. This could also be explained by the difference in molecular weights of the dyes and the cationic structure of rhodamine B. The interface between the two proteins is easier to observe when the dye is added to the sericin solution. The addition of dyes to the sericin layer did not show a large difference between the original and final location of interface. When the dyes were added to fibroin, movement of the dye into the sericin layer is relatively easy since sericin does not gel at 50°C. Conversely, when dyes were added to sericin, fibroin aqueous solution generates a three-dimensional polymer network during the gelation. This gel structure is to interfere with the penetration of the dye and facilitates the observation of the interface between fibroin and sericin.

In summary, the interface between fibroin and sericin could be monitored using azo dyes with a molecular weight high enough to prevent diffusion between the protein layers. It was also confirmed that when dyed sericin solutions were used, a more vivid interface was observed. This technique for monitoring the interface between fibroin and sericin using dyes could be helpful in understanding the interactions between these silk proteins.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2014-064335)

Reference

Bhatnagar, A., and Jain, A.K. (2005). A comparative adsorption study with different industrial wastes as adsorbents for the removal of cationic dyes from water. J. Colloid Interface Sci. 281, 49-55.

Jin, H.J., and Kaplan, D.L. (2003). Mechanism of silk processing in insects and spiders. Nature 424, 1057-1061.

Joosen, L., Hink, M.A., Gadella, T.W.J., and Goedhart, J. (2014). Effect of fixation procedures on the fluorescence lifetimes of Aequorea victoria derived fluorescent proteins. J. Microsc.. 256, 166-176.

Kundu, B., Rajkhowa, R., Kundu, S.C., and Wang, X. (2013). Silk fibroin biomaterials for tissue regenerations. Adv. Drug Deliv. Rev. 65, 457-470.

Lee, K.H. (2004). Silk sericin retards the crystallization of silk fibroin. Macromol. Rapid Commun. 25, 1792-1796.

Nisal, A., Trivedy, K., Mohammad, H., Panneri, S., Sen Gupta, S., Lele, A., Manchala, R., Kumar, N.S., Gadgil, M., Khandewal, H., *et al*. (2014). Uptake of Azo Dyes into Silk Glands for Production of Colored Silk Cocoons Using a Green Feeding Approach. ACS Sustain. Chem. Eng. 2, 312-317.

Oh, H., Kim, M.K., Lee, J.Y., Lee, K.H., (2009). The Role of Silk Sericin during the Spinning Process of Silk Fiber(I) -The Effect of Silk Sericin on the Crystallization and Gelation of Silk Fibroin- . Text Sci Eng. 46, 289-294

Okabayashi, R., Nakamura, M., Okabayashi, T., Tanaka, Y., Nagai, A., and Yamashita, K. (2009). Efficacy of Polarized Hydroxyapatite and Silk Fibroin Composite Dressing Gel on Epidermal Recovery From Full-Thickness Skin Wounds. J. Biomed. Mater. Res. Part B 90B, 641-646.

Tansil, N.C., Li, Y., Teng, C.P., Zhang, S., Win, K.Y., Chen, X., Liu, X.Y.,

and Han, M.-Y. (2011). Intrinsically Colored and Luminescent Silk. Adv. Mater. 23, 1463-1466.

Tansil, N.C., Koh, L.D., and Han, M.-Y. (2012). Functional silk: colored and luminescent. Adv. Mater. Weinheim 24, 1388-1397.

Tsukada, M., Obo, M., Kato, H., Freddi, G., and Zanetti, F. (1996). Structure and dyeability of Bombyx mori silk fibers with different filament sizes. J. Appl. Polym. Sci. 60, 1619-1627.

Wang, X., Kluge, J.A., Leisk, G.G., and Kaplan, D.L. (2008). Sonication-induced gelation of silk fibroin for cell encapsulation. Biomaterials 29, 1054-1064.

Wang, X., Yucel, T., Lu, Q., Hu, X., and Kaplan, D.L. (2010). Silk nanospheres and microspheres from silk/pva blend films for drug delivery. Biomaterials 31, 1025-1035.