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Dissolution of degummed *Antheraea yamamai* silkworm cocoon

You-Young Jo, Sung Min Bae, and HaeYong Kweon*

Sericultural and Apicultural Materials Division, National Institute of Agricultural Science, RDA, Wanju-gun, 55365, Republic of Korea

Abstract

Dissolution of *Antheraea yamamai* silkworm cocoon was carried out in various solvent systems with various dissolving conditions including dissolution salts, salt concentration, dissolving temperature, and time. General chaotropic salt for *Bombyx mori* silk fibroin does not work for *A. yamamai* silkworm cocoon. Lithium bromide 9.3 M at 100°C also does not work to dissolve wild silkworm cocoon. However, 9 M of lithium thiocyanate treatment at 100°C induced 100% dissolution of wild silkworm cocoon. But it could not be dissolved lower than 60°C. Like lithium thiocyanate, less than 60°C treatment with molten calcium nitrate 4 hydrate could not dissolve wild silkworm cocoon. As the dissolution temperature increased up to 100°C, the solubility of wild one was reached over 90%. SDS-PAGE showed broad tailing stream pattern that means the molecule of wild silk was depolymerized with dissolution temperature and time. From the above results, the best chaotropic salt for *A.yamamai* silkworm cocoon is calcium nitrate 4 hydrate.

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Introduction

Silk is classified into two general groups: domestic (*Bombyx mori*) and wild type (*Antheraea yamamai*, etc) (Kweon and Park, 1994). The general characteristics of *A.yamamai* silkworm silk were studied by my research group (Lee *et al.,* 2015; Kweon and Park, 1994). *A.yamamai* silk fiber has been used as a valuable textile fiber due to unique luster and color for more than 4,000 years. Recently, silk polymer has been revealed several advantages for biological materials due to its blood compatibility, oxygen permeability, and so on (Sakabe *et al.,* 1989; Minoura *et al.,* 1990). Silk polymer has been studied for various applications such as cosmetics, suture and artificial eardrum (Kundu *et al.,* 2013; Ju *et al.,* 2014; Vepari and Kaplan, 2007; Kim *et al.,* 2010), and so on. The reason why *B.mori* silk examined as possible biomaterials is that we know how to dissolve it and reshape it for the application. In the case of *A.yamamai* silk, it is known to have an arg-gly-asp tripeptide sequence (Ruoslahti and Pierschbacher, 1986, Minoura *et al.,* 1995). Biocompatibility (Wang *et al.,* 2011), gelation (Liu and Zhang, 2014), degradation (You *et al.,* 2014) and PEG modified film (Wei *et al.,* 2014) using *A.yamamai* silk protein have been investigated.

Until now there is no good solvent for silk materials because silk fibroin behaves like a thermoset polymer after solidification through spinning process. The only way to dissolve silk materials is concentrated chaotropic salts, which destabilize silk protein in solution and increase the solubility of fibroin (Kweon, 1998). The well known salts for dissolving silk fibroin are calcium chloride tertiary system, lithium salts, thiocyanate salts, and so on (Kim and Um, 2016; Kim *et*

***Corresponding author.**
HaeYong Kweon (Ph. D)
Sericultural and Apicultural Materials Division, National Institute of Agricultural Science, RDA, Wanju-Gun, Republic of Korea
Tel: +82-63-238-2872 / FAX: +82-63-238-3832
E-mail: hykweon@korea.kr

al., 2014; Kweon, 1998). Domestic silk fibroin is known to dissolve several chaotropic salts. But *A.pernyi* silk fibroin, a typical wild type silk polymer, has strong resistance to dissolve common calcium chloride tertiary system (Kweon and Park, 2001). The main conformation of *A. pernyi* silk fibroin is an extended antiparallel β-sheet structure of a hydrogen bond among the chains through –C=O and –NH groups. This intermolecular bonding is sufficiently strong to prevent the separation of the molecules and, hence, to resist their dissolution into various chemicals. According to Kweon's study, *A. pernyi* silk fiber can be dissolved in concentrated chaotropic salts including calcium nitrate solution (Kweon and Park, 2001; Lee *et al.,* 2013; Kweon *et al.,* 2003).

A.yamamai silk polymer is also one of abundant wild type silks and has been used as a valuable textile fiber. The chemical structure of *A. yamamai* silk is similar to that of *A. pernyi* silk. In this study, the dissolution method for *A. yamamai* silk polymer was examined with various chaotropic salts system.

Materials and methods

Materials

A.yamamai silkworm cocoons produced in National Institute of Agricultural Science, Wanju, Jeollabuk-do, Korea. Phosphate buffered saline (1X PBS, pH 7.4) was purchased from Life technology (California, U.S.A.). Acrylamide gel gradient 4-12% was purchased from Life technology from U.S.A. The ProsiBlue gel staining solution was purchased from GenDEPOT (Texas, U.S.A.).

Solubility

A.yamamai silkworm cocoon was degummed with alkali degumming method (Shin *et al.,* 2015; Lee *et al.,* 2015; Shin *et al.,* 2012). Briefly, the cocoon was degummed 3 times for 30 min under boiling water with Na_2CO_3 2.5g/L. And then the cocoon was dried under room temperature and then used as dissolution test.

To find the optimum dissolution condition for *A. yamamai* silk polymer, several dissolution parameters including salt types, salt concentration, treatment temperature, and time, were examined. The weight of the solid silk fibroin was filtered through non-woven fabrics and weighed after drying. The degree of dissolution was calculated with the following equation:

$$\text{Degree of solubility (wt\%)} = (W_i - W_f)/W_i \times 100$$

where, Wi is the initial weight of silk polymer (g), and Wf, the residual weight of the silkworm cocoon on the dried nonwoven fabrics (g).

SDS-PAGE

Polyacrylamide gel electrophoresis (PAGE) was performed according to Laemmli's method (1970) on 12.5% polyacrylamide gel containing 0.1% sodium dodecyl sulfate buffer. The proteins were stained with Coomasie Brilliant Blue R-250 (Sigma Chemical Co., St. Louis, MO)

Results and discussion

Dissolution using calcium chloride tertiary system

Calcium chloride tertiary system is well known solvent system for dissolving domestic silk (Kim and Um, 2016; Kweon, 1998). The author applied this system for *A.yamamai* silkworm cocoon and shown the results in Fig. 1. As expected domestic silk fibroin is easily solved in the system within 30 min, however, wild silkworm cocoon is not dissolved even though 180 min treatment.

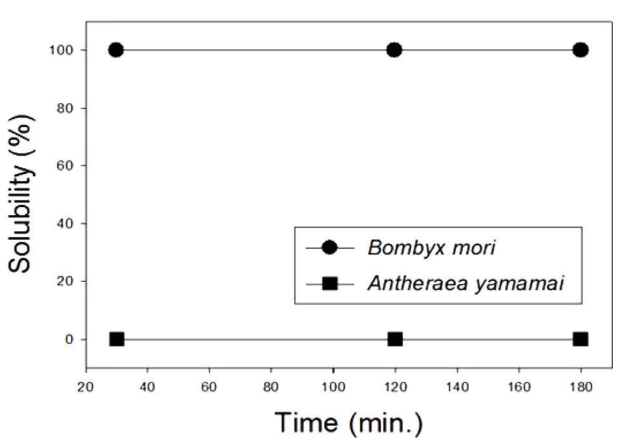

Fig. 1. Solubility of *Antheraea yamamai* silkworm cocoon treated with calcium chloride tertiary system.

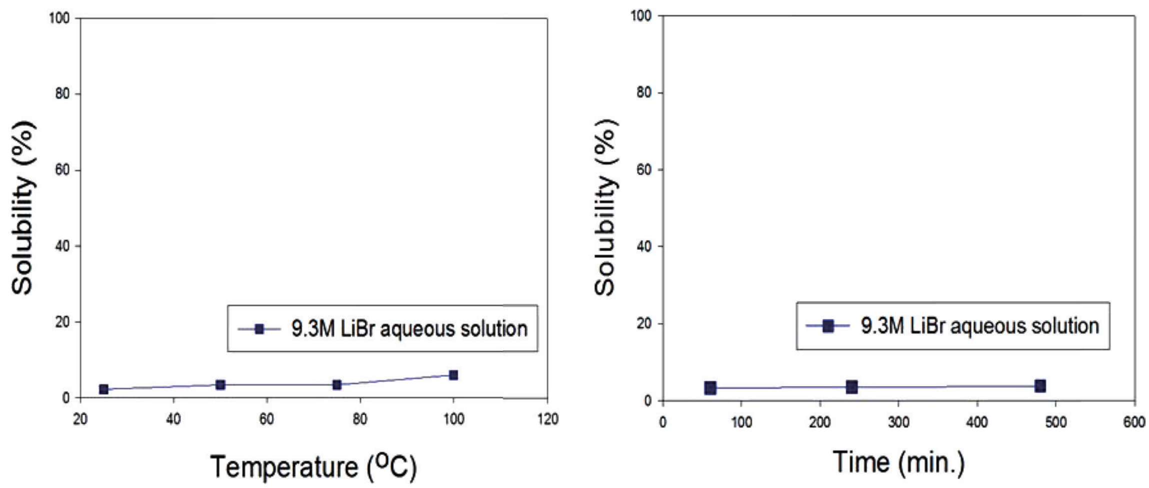

Fig. 2. Effect of treatment temperature (left) and time (right) on the dissolution of *Antheraea yamamai* silkworm cocoon treated with lithium bromide.

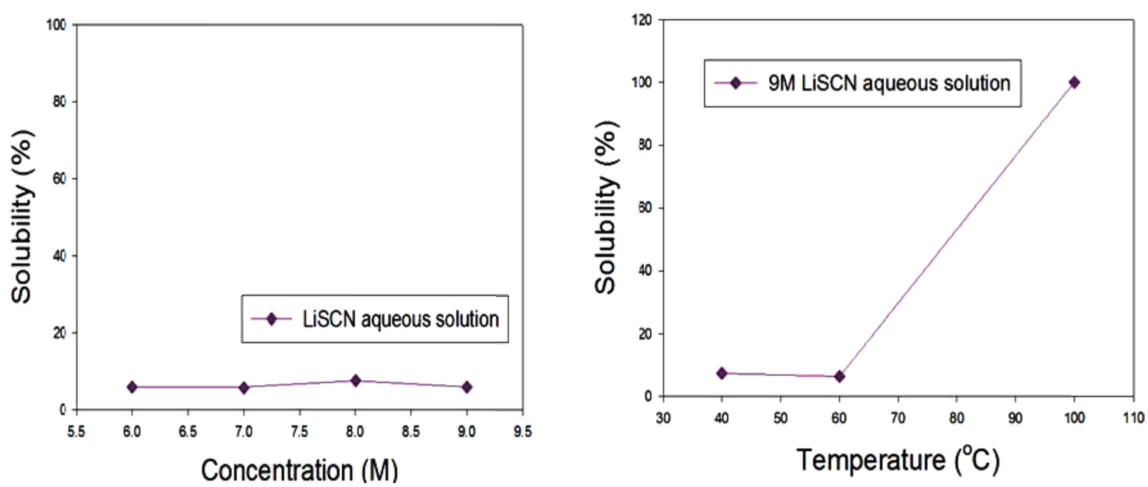

Fig. 3. Effect of treatment concentration (left) and temperature (right) on the dissolution of *Antheraea yamamai* silkworm cocoon treated with lithium thiocyanate.

Kweon *et al.* (1998) reported that similar wild silk spun by *A.pernyi* is also hardly dissolved in this system.

Lithium salt system

Lithium bromide is also used for dissolve *B.mori* silk fibroin (Cheng *et al.,* 2015). It is relatively mild and we can get natured form of silk materials (Kweon *et al.,* 2009). High concentrated lithium bromide was examined as solvent for *A.yamamai* silkworm cocoon. As shown in Fig. 2, the wild silkworm cocoon is hardly solved in the lithium bromide system. Although the author tried to extend the treatment time to 480 min to dissolve, the solubility of *A.yamamai* silkworm cocoon is not increased with time.

Lithium thiocyanate is one of powerful chaotropic salts for dissolving *B.mori* silk fibroin (Tsukada *et al.,* 1994). Fig. 3 shows the solubility of *A. yamamai* silkworm cocoon dissolved in various concentration of a lithium thiocyanate solution at 60°C for 3hr. Wild silkworm cocoon was nearly insoluble even thougth 9M lithium thiocyanate solution. However at the extreme condition at 9 M lithium thiocyanate and 100°C, the wild silkworm cocoon was dissolved completely.

Calcium nitrate system

Fig. 4 represents the solubility of *A. yamamai* silkworm cocoon dissolved in calcium nitrate 4 hydrate melt with temperature (60~120°C) and time (30~180 min). Less than 60°C,

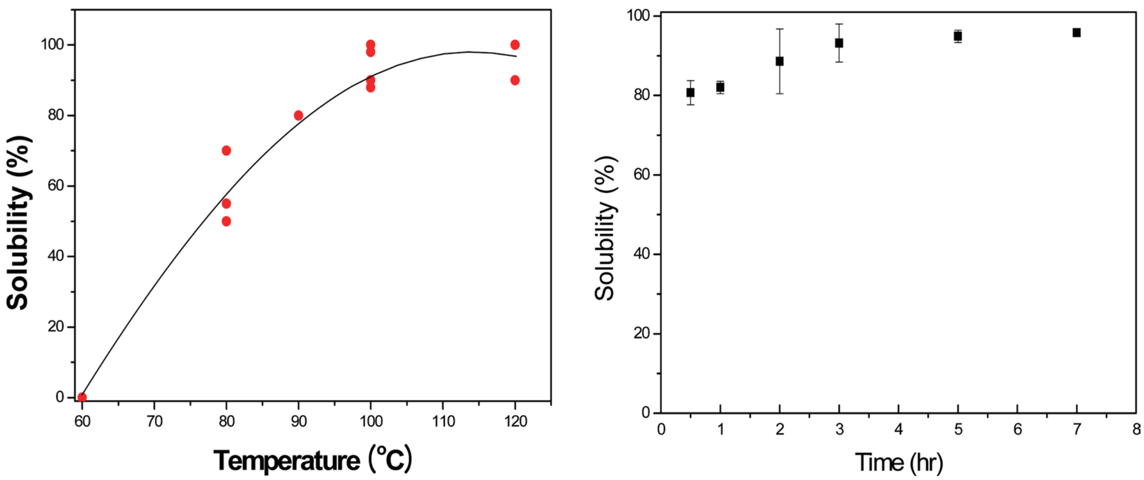

Fig. 4. Effect of treatment temperature (left) and time (right) on the dissolution of *Antheraea yamamai* silkworm cocoon treated with calcium nitrate.

the wild silkworm cocoon is hardly dissolved. However as the dissolution temperature increased up to 100°C, the solubility was increased linearly about 90 wt%. The solubility of *A.yamamai* silkworm cocoon dissolved 80% under molten calcium nitrate 4 hydrate at 100°C. From the above results, it is recommended that the dissolving conditions for *A. yamamai* silkworm cocoon is molten calcium nitrate 4 hydrate with 3 hr at 100°C.

Electrophoresis pattern of regenerated *A. yamamai* silk fibroin

SDS-PAGE has been used to elucidate the molecular weight of protein by many researchers. Fig. 5 shows the SDS electrophoresis pattern of regenerated wild silk fibroin. The stained pattern observed in Fig. 5 was lower with dissolution temperature and time.

Several researchers reported that the broad tailing stream of SDS-PAGE was to denaturation of silk polymer (Kim *et al.,* 2009; Aramwit and Sangcakul, 2007). These phenomena are a little different with that of *A. pernyi* dissolution (Kweon and Park, 2001). Electrophoresis pattern of *A. pernyi* showed several stained regions above 14 kDa, suggested mixture composition of molecular weight.

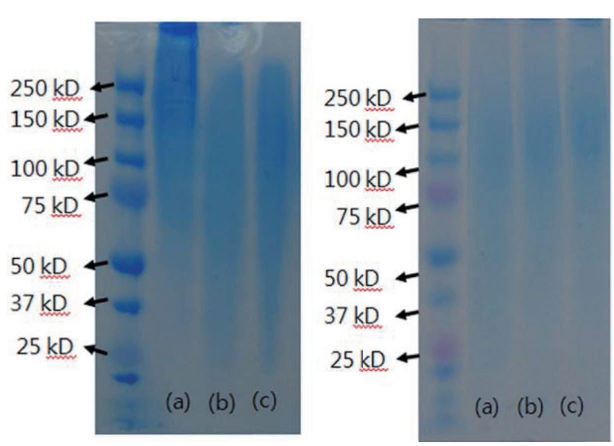

Fig. 5. SDS-PAGE patterns with temperature (left) and time (right)
Temperature (for 3 hr); (a) 80°C (b)100°C (c)120°C,
Time (at 100oC): (a) 3hr (b) 1hr (c) 0.5 hr

Therefore, it can be concluded that *A. yamamai* silkworm cocoon was depolymerized with dissolution time and temperature by scission of the main chains and resulted in the mixture composed of heterogeneous molecular weights.

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