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Sericinjam Sericin: Structural and Thermal Properties

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Structure and Properties of sericinjam sericin extracted was investigated using SDS-PAGE electrophoresis, UV, CD, and DSC. The molecular weight and its distribution of sericinjam sericin showed broad tailing pattern. Circular dichlroism spectra showed that the structure of sericinjam sericin in solution was different with domestic one. However, differential thermal calorimetric properties are similar to each other.

Key words: Sericinjam sericin, Circular dichroism, Ultraviolet absorption, Differential scanning calorimetry

Introduction

Silk sericin is one of major components of cocoon produced by silkworm. Sericin is known to be biosynthesized in the middle silk gland of the mature silkworm larva. Sericin is composed of 18 amino acids most of which have strongly polar side groups such as hydroxyl, carboxyl, and amine group. Now a day, sericin has been studied as one of new resources for non-textile materials such as cosmetics, food ingredients, pharmaceuticals, and so on (Oh *et al*., 2007, Kweon and Cho, 2001, Lee *et al*., 2001, Shin *et al*. 1997). Sericin has a good effect on the wound healing, no toxicity, and low inflammatory reaction (Aramwit and Sangcakul, 2007). Besides this properties, silk sericin has inhibition effect on the lipid peroxidation and tyrosinase activity (Kato *et al.,* 1998), UVB-induced acute damage and tumor promotion by reducing oxidative stress (Zhao *et al.,* 2003), anticoagulant activity of sulfated one (Tamada *et al.,* 2004). Sasaki *et al.* (2000) reported sericin enhances the bioavailability of Zn, Fe, Mg and Ca in rats. Therefore, many researchers have been studied to extract pure sericin from cocoon and degummed solution (Wu *et al.,* 2007, Dash *et al.,* 2006, 2007, Kim *et al.,* 2001, Fabiani *et al.,* 1996). National Academy of Agricultural Science, Suwon, Korea has been inbred sericinjam, which silkworm makes sericin-rich cocoon. The basic characteristics of sericinjam sericin were reported (Kweon *et al.,* 2009).

In this study the structural and thermal properties of Sericinjam sericin extracted by sodium carbonate was examined using various instrumental analysis.

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Materials and Methods

Sample collection

Cocoons of various inbred sericinjams were collected from National Academy of Agricultural Science, Suwon, Korea. The collected silkworm strain used in this study is shown in Table 1. The collected cocoons were kept in room temperature and were used for protein extraction.

Isolation of sericin from the cocoon

Sericin was isolated from sericinjam cocoons. In brief, the fresh cocoon was dissolved in 0.5% on the weight of fiber (o.w.f.) sodium carbonate solution at 100°C for 30 min twice and then filtered with nonwoven fabric (MiraCloth).

Table 1. Inbred silkworm strain used in this study and extraction rate

Sample number	1	2	3	4	5
Silkworm strain	DM458× C522	C212× Jam307	DM458	Jam307× P50	Baekok-jam
Extraction rate (%)	99.6	99.8	100	100	43.0

The filtered solution was dialyzed in semi-permeable cellulose membrane (MWCO = 3,500) to remove the dissolving salt for 3 days.

Extraction rate (wt.%) = (Wi−Wf)/Wi

Where, Wi is the initial weight of sericinjam cocoon (g), and Wf, the residual weight of the sericinjam cocoon.

Characterization

To determine the molecular weight of sericin extracted from sericinjam SDS-PAGE analysis was performed according to Laemmli (1970). The extracted proteins were resolved by 10% preparative SDS-PAGE under non-reducing condition. And stained in Coomassie Blue R-250 and then washed thoroughly.

The ultra-violet absorption spectra were obtained from UV/VIS spectrometer (Lambda 10, Perkin Elmer, USA).

Circular dichroism (CD) spectra were measured using a Jasco J-715 spectrometer equipped with a quartz cell having a pathlength of 10 mm at room temperature.

Differential Scanning Calorimetric curves were obtained through differential scanning calorimeter (TA Instrument, TA 2910, UK) at a heating rate of 10°C/min and nitrogen gas flow rate of 50 ml/min.

Results and Discussion

Dissolution and isolation of sericin

Sericin was dissolved in sodium carbonate solution and calculated the extraction rate from cocoon weight change. The cocoon of sericinjam was over 99%, and that of control was 42%. Hot water extracted over 96% of sericinjam, on the other hand 24% of domestic sericin.

Molecular weight of sericin

SDS-PAGE has been used to examine the molecular weight of protein. Electrophoretic analysis of the purified sericin in 10% SDS-PAGE showed broad tailing stream (Fig. 1). Previous report (Kweon *et al.*, 2009) showed that the molecular weight of sericinjam sericin extracted with LiBr was determined to be approximately 250 kDa, 120 kDa, 90 kDa, 70 kDa, and 40 kDa regardless of the different inbred silkworm strain. Gamo *et al.*, (1977) reported that sericin is a complex mixture of 5~6 polypeptides differing widely in size 40~400 kDa. Sericin solution without heat treatment (Takasu *et al.*, 2002) exhibited distinct bands of three main sericin components at >250, 180, and 100 kDa. However, the SDS patterns of sericin extracts are completely different with our previous results [Kweon et al 2009] due to the difference of extraction method. The molecular weight of silk sericin extracted with LiBr solution shows clear distinct bands, however

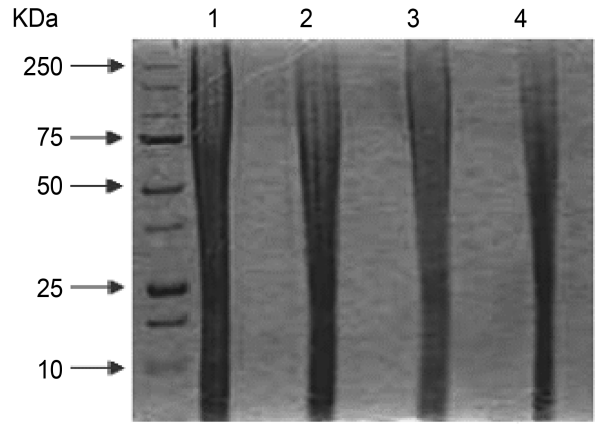

Fig. 1. SDS-PAGE analysis of sericinjam sericin. 1, DM458 ×C522; 2, C212 × Jam307; 3. DM458; 4, Jam307 × P50.

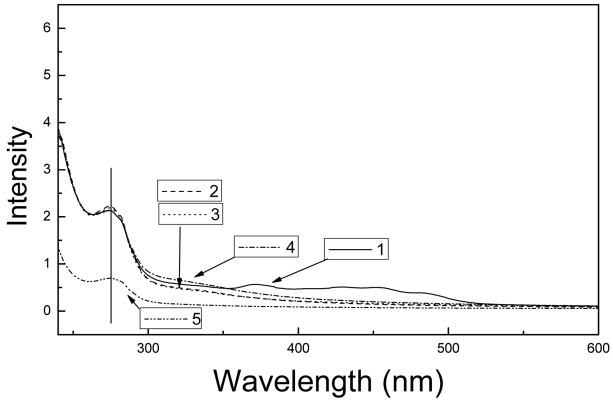

Fig. 2. UV-VIS spectra of sericinjam sericin. 1, DM458 × C522; 2, C212 × Jam307; 3. DM458; 4, Jam307 × P50; 5, Baekokjam.

that extracted with sodium carbonate showed broad stained pattern due to the mixture of different molecular weight peptides. Aramwit and Sangcakul (2007) reported that similar results extracted by autoclaving method. According to the above research results, the molecular weight and its distribution silk sericin was varied with the denaturalization methods.

UV spectra of sericin

Sericin is one of a protein synthesized by silkworm. Generally, proteins absorb near-ultraviolet region due to the electron transfer of aromatic amino acid, tryptophan, tyrosine, and phenylalanine. On the other hand, histidine absorbs at 210 nm, far-ultraviolet region. Seicinjam sericin be expected also the absorption at 280 nm, near ultraviolent region. As expected (Fig. 2), sericinjam sericin showed strong absorption band at 280 nm. But sericinjam sericin extracted from DM458×C522 strain showed broad absorption band at around from 350 to 520 nm, due to the color of sericinjam cocoon.

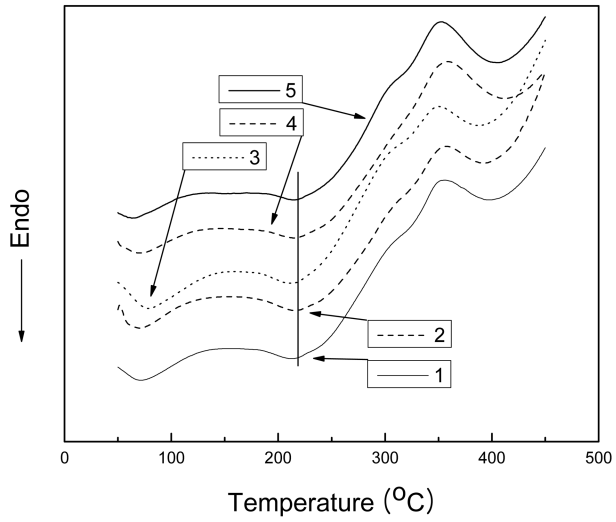

Fig. 3. CD spectra of sericinjam sericin. 1, DM458 × C522; 2 C212 × Jam307; 3. DM458; 4, Jam307 × P50; 5, Baekokjam.

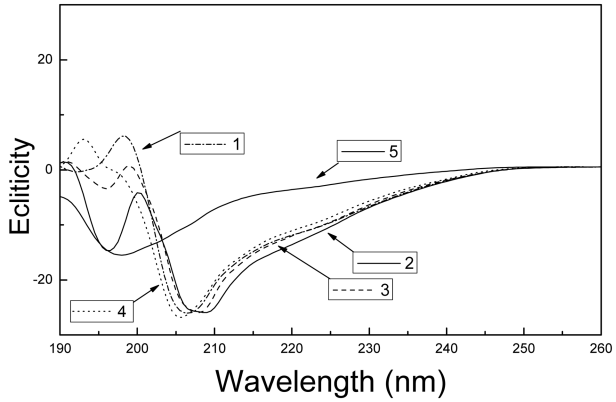

Fig. 4. DSC curves of sericinjam sericin. 1, DM458 × C522; 2, C212 × Jam307; 3. DM458; 4, Jam307 × P50; 5, Baekokjam.

Circular dichroism analysis of sericinjam sericin

CD spectra of sericinjam sericin are shown in Fig. 3. CD spectrum of domestic silk sericin showed a peak at 198 nm, an indication of a random coil conformation (Kweon *et al.,* 2000 1302). However, sericin extracted from sericinjam cocoon showed a little different pattern. They showed some positive bands at around 192~200 nm and the strong negative band at around 205~209 nm with different sericinjam strain. The CD spectra of sericin added by methyl alcohol were changed from 198 nm to 205 nm, because of the change of molecular environment of silk sericin (Lee *et al.,* 2003). The negative peak at around 208 nm is attributed to the α-helix structure (Tsukada and Hirabayashi 1983). In the case of domestic and wild silk fibroin, alcohol induces the conformational changes of silk fibroin solution from random coil to β-sheet (Nam and Park 2001, Kweon *et al.,* 2000, Canetti *et al.,* 1989). Beta-sheet structure of silk sericin exhibits a strong negative band at a 218 nm (Kweon *et al.,* 2000. Towell 1994). Therefore, the results indicated that the conformation of sericinjam sericin was different with that of domestic sericin in aqueous solution. Sericinjam sericin has helical structure in aqueous solution because helical structure is more stable than random coil structure.

Differential thermal scanning calorimetric analysis

Thermal behavior measured by DSC is closely related to the structural characteristics of silk materials. Differential thermal scanning calorimetric characteristics of sericinjam sericin are shown in Fig. 4. In general, thermal decomposition temperature of silk fiber is about 350°C, which is detected in the case of well-oriented fiber containing β-sheet structure (Kweon and Park, 1994). According to our results, sericin showed a broad endothermic peak at around 220°C which is characteristics of easy soluble sericin (Kim *et al.,* 2001). It is quite different with the sericin extracted by LiBr (Kweon *et al.,* 2009). The previous work reported that three endothermic peaks at 220°C, 255°C and 310°C attributed to the characteristics of decomposition of sericin.

We investigated sericin extracted from sericinjam, inbred at National Academy of Agricultural Science, Suwon, Korea. The structure and properties of sericinjam sericin were varied with the extraction methods.

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