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Characteristics of Silk Sericin Extracted from Sericinjam

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We investigated sericin extracted from sericinjam, which was inbred at National Academy of Agricultural Science, Suwon, Korea. Sericinjam sericin is composed of 5 fractions: 250 kDa, 120 kDa, 90 kDa, 70 kDa and 40 kDa. Amino acid analysis showed that the major amino acids of sericinjam sericin were Ser, Gly, Asp, Glu, Thr and Ala. Infrared spectra showed that sericinjam sericin has β-sheet structure. Thermal property of sericin was investigated using DSC and then they showed characteristic degradation peak at around 215~240°C.

Key words: Sericinjam sericin, Amino acid, β-sheet structure, Degradation

Introduction

Silk sericin is one of major components of cocoon produced by silkworm. Sericin is biosynthesized in the middle silk gland of the mature silkworm larva and constitutes 25-30% of silk protein. Sericin is composed of 18 amino acids most of which have strongly polar side groups such as hydroxyl, carboxyl, and amino acids. Sericin envelops and protects the fibroin fiber with successively layer. In general, sericin was removed from silk yarn to improve the quality of silk fabric. But, now a days, sericin has been studied as one of new resources for non-textile materials such as cosmetics, food ingredients, pharmaceuticals, and so on (Kweon and Cho, 2001; Lee *et al*., 2001; Oh *et al*., 2007). 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).

Generally, most silkworms cocoon with fibroin and sericin. Recently new silkworm varieties with specific purpose have been bred in National Academy of Agricultural Science, RDA, Korea (Kang *et al*., 2004). Sericinjam, silkworm cocoons with sericin, has been breeding through systematic hybridization to obtain pure sericin without other treatment. In this study we extracted and characterized silk sericin from sericinjam.

Materials and Methods

Sample collection

Cocoons of various inbred sericinjams were collected from the 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 the 8 M LiBr solution for 24 hrs. After adjusting the pH to 8 with 1 M Tris-HCl buffer, the solution was dialyzed in semi-permeable cellulose membrane (MWCO=3,500) to remove the dissolving salt for 3 days. Extraction rate of sericin was calcu-

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Table 1. Inbred silkworm strain used in this study

Sample number	1	2	3	4	5
Silkworm strain	DM458×C522	C212×Jam307	DM458	Jam307×P50	Domestic Silkworm (Baegokjam)

lated by the weight changes of sericinjam cocoon.

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, stained in Coomassie Blue R-250 and then washed thoroughly.

Amino acid composition analysis was carried out using Biochrom 20 Amino Acid Analyser (Amersham Pharmacia Biotech. Co., Sweden). The 10 mg of samples were hydrolyzed in 6 N HCl at 110°C for 18 hrs. The filtrate was loaded on the analyzer after 0.2 um PVDF Acrodisc LC 13 syringe filter.

Fourier transform infrared spectra were obtained using FT-IR spectrometer (Spectrum 100, Perkin Elmer, USA) in the spectral region of 1400~500 cm^{-1}.

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 8 M LiBr solution and calculated the extraction rate from cocoon weight change. The dissolution rate of sericinjam was increased from ca. 15% (35°C) to 75% (70°C) with dissolution temperature.

Molecular weight of sericin

SDS-PAGE has been used to elucidate the molecular weight of protein by many researchers. Electrophoretic analysis of the purified sericin in 10% SDS-PAGE showed 5 bands (Fig. 1). The molecular weight of each band 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. Takasu *et al.*, (2002) reported

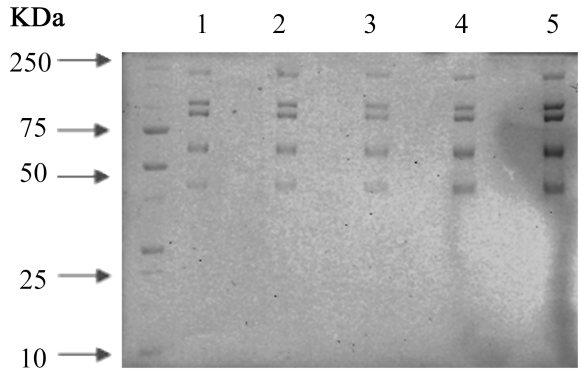

Fig. 1. SDS-PAGE analysis of sericin from sericinjam. 1, DM458×C522; 2, C212×Jam307; 3, DM458; 4, Jam307×P50; 5, domestic silkworm (Baegokjam).

that sericin solution without heat treatment exhibits distinct bands of three main sericin components at >250, 180, and 100 kDa. Aramwit and Sangcakul (2007) reported that the sericin extracted by autoclaving method showed broader bands due to the mixture of different molecular weights peptides. According to the above research results, the molecular weight of silk sericin was varied with the denaturalization methods. However, the molecular weight of sericin, in this study, showed the similar molecular weights in spite of the different silkworm strains of sericinjam and domestic silkworm, *B. mori*.

Amino acid composition of sericinjam cocoon

Amino acid composition of sericinjam cocoon is represented in Table 2. The major amino acids of sericinjam are different with those of domestic silkworm. The major amino acids of sericinjam are: Ser, Gly, Asp, Glu, Thr and Ala, but those of domestic cocoon are Ser, Gly, Asp, Ala, Glu, and Thr. Table 3 shows the comparison of selected amino acids for sericinjam cocoons. The content of hydrophilic amino acids of sericinjam cocoon including OH-containing amino acids and acidic and basic amino acids are higher than those of domestic cocoon. The polar/non-polar amino acids ratio of inbred sericinjam cocoon is over 1.6 times.

Many silk researchers often investigate the conformation of silk polymer using FTIR spectroscopy since the specific absorption bands of IR spectrum are sensitive to the molecular conformation of silk protein. The IR spectra of sericinjam sericin freeze-dried are shown in Fig. 2. Domestic sericin (Fig. 2 (5)) shows an absorption band at

Table 2. Amino acid composition of sericinjam and domestic cocoon

sample	**Asp**	Thr	**Ser**	Glu	**Gly**	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg
1	**12.5**	7.8	**18.0**	8.1	**13.4**	6.3	3.9	–	1.2	1.3	2.6	0.5	1.5	3.5	3.6
2	**12.6**	8.0	**16.0**	8.1	**13.5**	6.6	4.3	–	1.2	1.4	2.7	0.5	1.9	3.8	3.6
3	**12.5**	7.2	**18.8**	7.4	**12.8**	5.7	3.7	–	1.1	1.2	2.2	0.4	1.6	3.3	3.1
4	**12.5**	8.1	**17.0**	8.5	**13.3**	7.3	4.2	0.2	1.1	1.3	3.7	0.5	1.7	3.6	3.7
5	**12.1**	7.2	**17.6**	7.3	**14.6**	7.6	4.0	–	1.3	1.5	1.8	0.5	1.3	3.0	3.2

Table 3. Comparison of selected amino acids for sericinjam cocoons

sample	OH-containing amino acid	Acidic amino acid	Basic amino acid	P/NP ratio
1	28.4	20.6	8.6	1.6
2	26.7	20.7	9.3	1.6
3	28.2	19.9	8.0	1.7
4	28.8	21.0	9.0	1.6
5	26.6	19.4	7.5	1.4

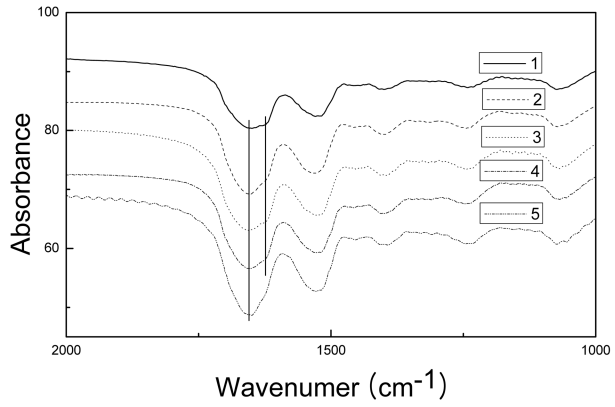

Fig. 2. FT-IR spectra of sericinjam sericin. 1, DM458×C522; 2, C212×Jam307; 3, DM458; 4, Jam307×P50; 5, domestic silkworm (Baegokjam).

around 1655 cm^{-1} (amide I), indicating random coil conformation (Kweon *et al.*, 2000; Lee *et al.*, 2002, 2003). On the other hand, sericinjam sericin (Fig. 2 (1-4)) showed strong absorption bands at around 1655 cm^{-1} and shoulder peak at around 1625 cm^{-1}, which was attributed to β-sheet conformation. This is very interesting results. Teramoto *et al.* (2007) reported *B. mori* silk sericin formed β-sheet aggregates after hydration. Especially hydroxyl side chains of Ser and Thr residues in β-sheet structure remain rigid after hydration. The reason why the lyophilized sericinjam sericin showed random coil conformation with β-sheet structure is not clear. But we might explain that the sericinjam sericin itself should be stable against environment around cocoon to protect larva. Therefore, they needs relatively stable structure compared with domestic silkworm sericin.

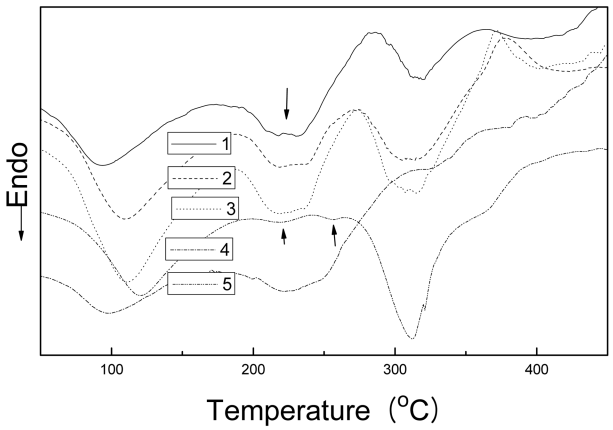

Fig. 3. Differential scanning calorimetric curves of sericinjam cocoon. 1, DM458×C522; 2, C212×Jam307; 3, DM458; 4, Jam307×P50; 5, domestic silkworm (Baegokjam).

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 cocoon are shown in Fig. 3. 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 (Fig. 3 (5)), domestic silkworm cocoon showed a water evaporation peak at around 100°C and 3 endothermic peaks at around 220°C, 255°C, and 310°C. The former two peaks are attributed to the decomposition of easy and difficult soluble sericin. On the other hand, sericinjam cocoon shows a water evaporation peak at around 100°C and a major degradation peaks around 215-240°C. Sericin extracted with hot water showed endothermic peaks at 216°C and 313°C (Kim *et al.*, 2001; Lee *et al.*, 2002). And sericin extracted from degummed solution showed 220°C, 270°C, and 325°C (Kim *et al.*, 2001).

We investigated sericin extracted from sericinjam, which was inbred at National Academy of Agricultural Science, Suwon, Korea. Sericinjam sericin is composed of 5 fractions: 250 kDa, 120 kDa, 90 kDa, 70 kDa, and 40 kDa. Their primary and secondary structure was examined and then they are more polar and more stable than domestic

one. As new resources of silk polymer further study should be carried out.

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