# 繭糸セリシンの接着性と構造の関係


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# Relationship between adhesive properties and structure of sericin in cocoon filaments

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The structure of sericin is related to the stripping resistance of cocoon filaments. When it was strong, the thermal absorption peak of DSC curve for sericin appeared at higher temperature region, indicating that both $\beta$-structure and random coil structure were present in sericin. On the other hand, when the stripping resistance was weak, sericin had only the random coil structure. The adhesive strength of sericin closely depended on the phase of sericin, being strong when the sericin molecule in solution transformed from the random coil to the $\beta$-structure. This was also true when cocooning was done at high temperature and high humidity. These results demonstrated that adhesion of sericin to cocoon filaments was dependent on the sericin structure.

Key words: sericin, cocoon filament, stripping resistance, adhesive strength, gelation, $\beta$-structure

The cocoon filament has the 2 components, a fibrous component fibroin and a gelatinous component sericin. Sericin provides a cementing layer on the surface of the filament to keep the cocoon intact. Thus the filament from a cocoon cannot be unwound unless sericin is reswollen and softened. Adhesion of sericin closely reflects the atmospheric conditions during cocooning; the cocoon filament is difficult to reel when the cocoon is spun at high temperature, high humidity and low air flow. Under these atmospheric conditions, sericin sticks hardly on the surface of filament and, as a result, the solubility of sericin reduces and the reeling tension increases. The increase of reeling tension causes excessive dropping of filaments during reeling (UEDA and SUZUKI, 1976; HUANG, 1983). Solubility of sericin in water was found to be correlated with the structure of sericin, decreasing when the sericin molecules are transformed from random coil into the $\beta$-structure and crystallized state (KOMATSU, 1975). This transformation is influenced by the moisture content of sericin (KATAOKA, 1977a). Hence, the atmospheric conditions for cocooning are the most important factor affecting the changes of sericin properties. Spinning of cocoon filament by a matured larva under conditions of

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high humidity changes remarkably the molecular structure of sericin.

The present article deals with the stripping resistance of filaments, the molecular structure of sericin and its thermal decomposition studied by using the cocoons which were spun under different atmospheric conditions. Also studied were the adhesive properties of sericin in connection with the stripping resistance of cocoon filament. In addition, the relationships of the adhesive strength and structure of sericin to the phase of sericin were analyzed.

## Materials and Methods

*Reeling of cocoons and preparation of sericin solution and sericin powders*: Matured larvae of a race, Asahi × Tokai, of *Bombyx mori* were allowed to spin cocoons under different atmospheric conditions. At the H-zone the temperature and humidity were as high as 30℃ and 94% RH, respectively, and at the N-zone the temperature and humidity were normal (23℃, 67% RH). The sericin solution was prepared from cocoon pieces as described in a preceding paper (ZHU *et al.*, 1995). To obtain sericin powers, the solution was dried in air at 25℃.

*Physical and morphological analyses of sericin*: The presence of sericin in cocoon filaments was studied with an electron microscope (Hitachi, H-700H). Using 30 dried cocoons cooked in a boiling water bath, the stripping resistance of filaments was determined with the top end of the cocoon filament being held to a tensionmeter (Tensilon, UTM-II). Measurements were done at a pulling speed of 40 mm/min and a recording speed of 200 mm/min. Sericin powders (4 mg) were subjected to DSC analysis in air with a thermal analyzer (Shimadzu, DT-30) at a heating rate of 20℃/min and a sensibility of 4 mcal/sec. The sericin solution was adjusted to 15 μg/ml with distilled water and analyzed for the circular dichroism (CD) spectrum with a JASCO J-700 instrument at wavelengths of 190 to 240 nm. The inserting resistance of 1.0% sericin solution and gel in a beaker was measured by inserting a modified probe with a

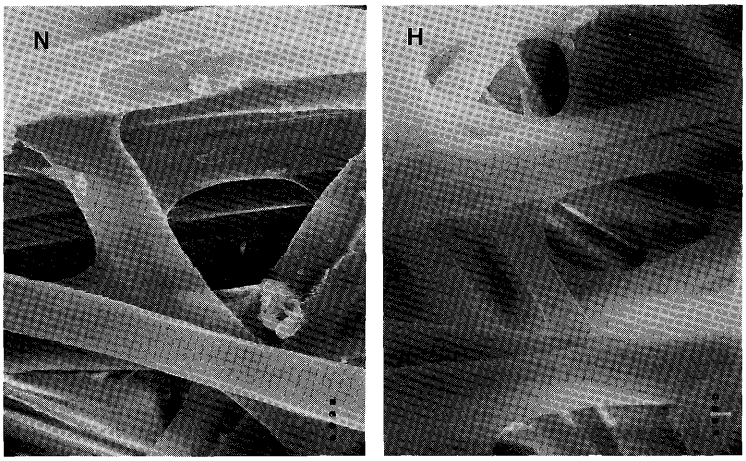


Fig. 1. Electron micrographs of cocoon filaments from different treatment zones. H, at 30℃ and 94% RH; N, at 23℃ and 67% RH. Bar = 5 μm.

diameter of 8 mm (ZHU *et al.*, 1995). To measure the adhesive strength, 1.0% sericin solution (100 $\mu$l) was uniformly paved on a silk cloth (20 × 10 mm$^2$), which was then covered with silk filaments. Then the complex was dried in air at 25 ℃ and applied to a tensionmeter at a pulling speed of 40 mm/min and a chart speed of 200 mm/min. The sericin solution was kept at 25℃ for indicated periods of time, dried at 50℃ and observed for a morphological feature of sericin using a Nikon AFM light microscope. Also the structure of sericin was studied using an infrared (IR) spectrophotometer (Shimadzu, IR-435).

## Results and Discussion

The electron micrographs of cocoons spun under different atmospheric conditions (in the H and N zones, see Materials and Methods) show the presence of sericin in the space between filaments (Fig. 1). Filaments were in close contact to each other due to the adhesiveness of sericin. The adhesive area of sericin from the H-zone was larger than that of sericin from the N-zone.

The stripping resistance measured after cooking of cocoon filaments from the H-zone was stronger than that of the counterparts from the N-zone (Table 1). These results suggest that the stripping resistance was increased due to the changes in adhesion of sericin during cocooning at high temperature and humidity, making reeling of cocoon filaments very difficult.

Table 1. Stripping resistance of cocoon filaments from the cocoons spun at different treatment zones.

| Range | Stripping resistance (N/mm$^2$) | |
|---|---|---|
| | H (30℃, 94% RH) | N (23℃, 67% RH) |
| max. | 10.88±1.18 | 3.82±0.78 |
| min. | 8.62±1.08 | 2.65±0.49 |

As a measure for the thermal properties of sericin, DSC was analyzed with sericin powders (Fig. 2), wherein those from both cocoons spun at the H and N zones showed 3 absorption peaks, although their shapes and heights were different. The 1st peak for all sericin specimens appeared around 110 ℃, indicating the vaporization of water in sericin. The 2nd peaks at 218℃ for the sericin from the N-zone and at 230℃ for that from the H-zone indicated the thermal decomposition, which occurred probably for mutual slides in sericin molecules. The 3rd peaks appeared at 268 and 255℃ for the specimens from the H and N-zones, respectively. The former peak was sharper than the latter one. This thermal decomposition behavior of sericin was in accordance with previous results (HIRABAYASHI *et al.*, 1976). When the solubility of sericin was low, the thermal decomposition peak shifted to the higher temperature side,

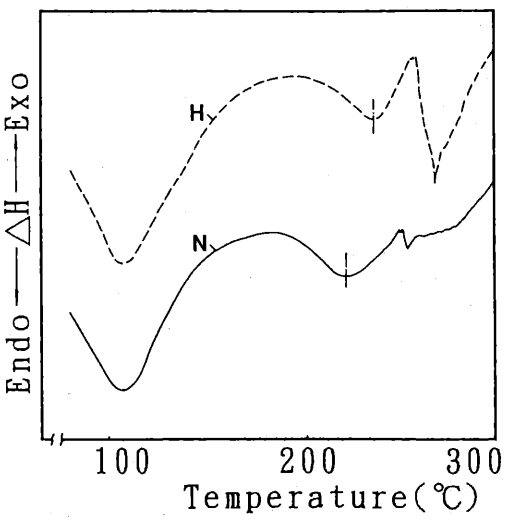


Fig. 2. DSC curves of sericin prepared from cocoon filaments from different treatment zones. H, at 30℃ and 94% RH; N, at 23℃ and 67% RH.

and, as a result, the stripping resistance became stronger. On the other hand, the thermal decomposition peak of sericin with an improved solubility shifted to the lower temperature side.

CD analysis was carried out to assess the transformation in molecular structure of sericin on the basis of the locations of absorption peaks (Fig. 3). Negative absorption peaks for the specimens from the H- and N-zone appeared at 198 and 200 nm, respectively, and a negative peak at 218 nm only appeared for that from the H-zone. As to the $\beta$-structure the positive peak must be in the range of 195 to 197 nm and the negative peak 217 to 218 nm, whereas as to the random coil the negative peak must be in the range of 196 to 202 nm and the positive peak 215 to 218 nm (HOLZWARTH *et al.*, 1962; FASMAN and DAVIDSON, 1968; HIRABAYASHI, 1980). From these criteria, the present results suggested that the sericin from the H-zone contains both $\beta$-structure and random coil, and the sericin from the N-zone contains only the random coil structure.

The sericin specimens dissolved from cocoon filaments by boiling for the initial 30 min must originate from the outer portions. Since the outer sericin covers the surface of cocoon filaments, the stripping resistance of cocoon filament is considered to have a close relation with the outer layer sericin. Thus the transformation of sericin structure is an essential factor in sericin adhesion. On the other hand, the stripping resistance of cocoon filament had a strong connection with the secondary structure of sericin, being stronger when they were spun under the conditions of higher temperature and higher humidity. These facts indicate that the molecular structure of cocoon sericin was affected by both temperature and humidity during cocooning.

The sericin solution was slowly transformed into a gel after extracted from the cocoon filaments. Fig. 4 shows the CD spectra of sericin solution and gel. A negative absorption peak appeared only at 198 nm for the

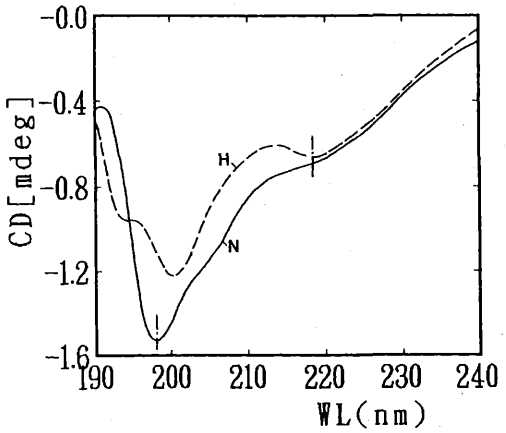


Fig. 3. CD spectra of sericin solution prepared from cocoon filament from different treatment zones. H, at 30℃ and 94% RH; N, at 23℃ and 67% RH.

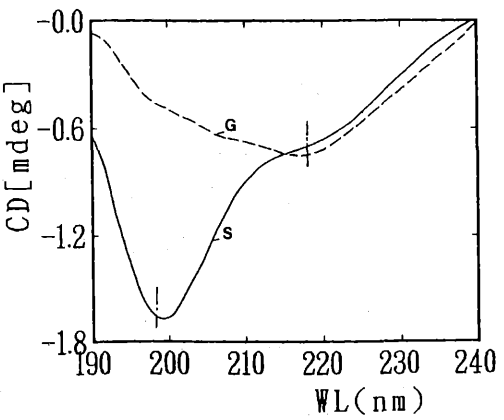


Fig. 4. CD spectra of sericin solution and gel. S, solution; G, gel.

solution, but peaks appeared at 198 and 218 nm for the sericin gel. The results imply that the sericin solution has only the random coil structure, while the sericin gel had both β-structure and random coil structure.

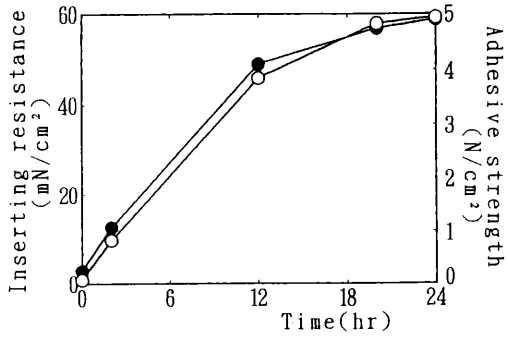


Fig. 5. Changes in inserting resistance and adhesive strength of sericin. Solid circles, inserting resistance of sericin during gelation; open circles, adhesive strength of sericin coated on a silk cloth.

Fig. 5 shows the changes in inserting resistance and adhesive strength of sericin at 25℃. The inserting resistance of sericin was changed with time, showing a maximum when gelation of sericin was completed after 24 hr. The adhesive strength of sericin coated on a silk cloth was increased with time. Therefore, the resistance or strength of sericin is likely to be increased with the gelation of sericin. This result demonstrated that the sericin from the H-zone had a similar structure to that of the sericin gel, which had both β-structure and random coil with a strong stripping resistance or strength. On the other hand, the sericin from the N-zone had a structure like the sericin solution with only the random coil structure and the weak stripping resistance or strength.

Light microscopical appearance of the sericin solution after incubation at 25℃ for different times (followed by drying, see Materials and Methods) changed as shown in

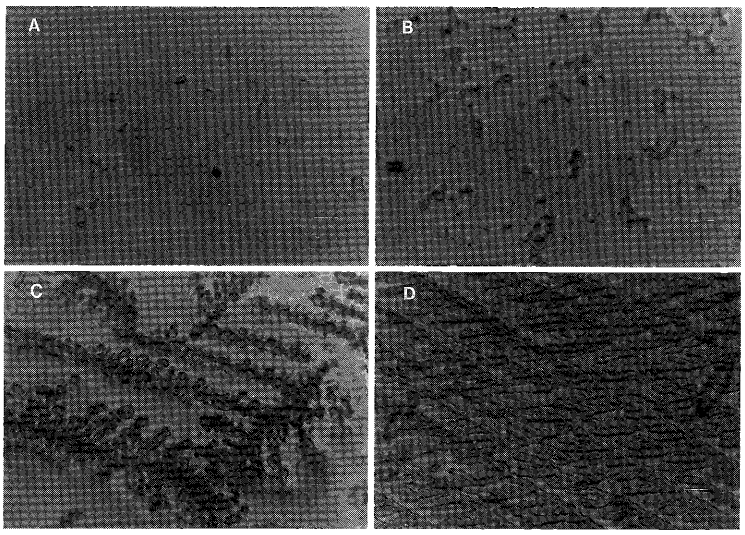


Fig. 6. Photographs of sericin kept for different times at 25℃. A, 0 hr; B, 2 hr; C, 3 hr; D, 12 hr. Bar = 5 μm.

Fig. 6. At 0 hr of the incubation, the specimen exhibited granules. At 2 hr sericin crystals were seen; these were similar to the initial stage of dendric crystals. Sericin kept for more than 3 hr exhibited dendric or lamella crystals. The appearance of sericin was thus very different at the 4 different observation times. IR analysis indicated that the molecular structure of sericin in dendric crystals had the $\beta$-structure (Fig. 7). The sericin under these conditions might have also a granular random coil structure (KOMATSU, 1975; KATAOKA, 1977b). It should be noted that gelation of sericin occurs when treatment time is long. The adhesive properties such as adhesive strength of sericin is closely related to the phase of sericin, becoming strong when sericin solution was turned to a gel with the formation of $\beta$-structure.

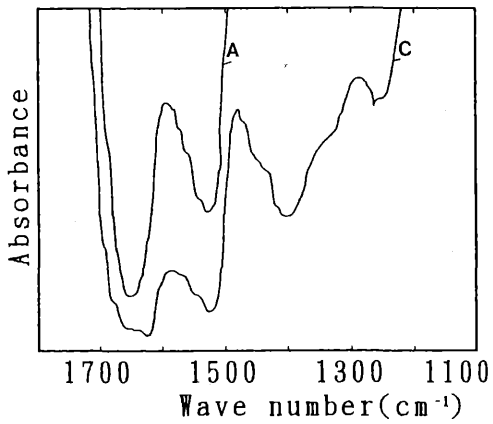


Fig. 7. IR spectra of sericin kept for 0 hr (A) and 3 hr (C) at 25℃.

The adhesive properties and molecular structure of sericin are chiefly affected by the atmospheric conditions for cocooning. These facts are summarized in Fig. 8. When sericin was spun at normal temperature and normal humidity the structure was random coil, and the stripping resistance of cocoon filament was weak. Similarly the adhesion of sericin was weak. On the other hand, the spinning at high temperature and high humidity transformed the molecular structure of sericin from the random coil to the $\beta$-structure. Thus the stripping resistance of cocoon filament and the adhesion increased. The gelation of sericin helped increase the adhesive strength by the structural transformation at high temperature and high humidity.

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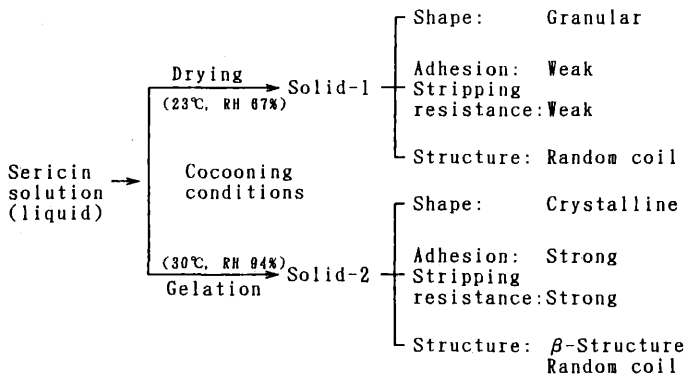


Fig. 8. Explanation of adhesive mechanism of sericin on cocoon filaments.

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朱 良均・荒井三雄・平林　潔: 繭糸セリシンの接着性と構造の関係

セリシンの構造は繭糸の剥離抵抗に関係する。繭糸剥離抵抗が強くなると，セリシンの熱分解温度は高温側へシフトし，分子構造は$\beta$型とランダムコイルが混在するのに対して，繭糸剥離抵抗が弱いセリシン分子にはランダムコイルだけ存在していた。セリシンの接着強度はセリシンの状態に依存していた。高温多湿の条件下で上蔟した繭糸のセリシンでは分子構造はランダムコイルから$\beta$型への転化が進んで，セリシンの接着強度が強くなっていた。繭糸セリシンの接着性はセリシンの分子構造に依存していた。