SHORT COMMUNICATION

Conformation of Liquid Silk Sericin

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Raw silk fibers consist of mainly two proteins: silk fibroin and silk sericin; the latter being secreted from the middle silk gland of the silkworm. The liquid silk sericin whose content is 25 wt% of the cocoon filament, forms the skin layer of the silk fiber, while the inner core is constituted by fibroin. Since silk fibroin is the major constituent of silk thread, it has been widely studied.¹⁻³ We are interested in the conformation of silk sericin, the amino acid composition of which was examined by Shimizu, *et al.*⁴ Studies were also made on conformation,⁵ molecular weight,⁶ physicochemical properties,⁷ and crystallization⁸ during the secretion of silk sericin. However, the amino acid sequence and the supermolecular structure remain unsolved. It may be worthwhile to estimate quantitatively the conformations of silk sericins in relation to their amino acid compositions. This communication presents a conformational study of silk sericin which was studied in the course of spinning from the moth *Nd-s* silkworms.

EXPERIMENTAL PROCEDURES

Liquid sericins of silkworms (*Nd-s* and *bombyx mori*) were collected from the silk gland and extracted by water for 5 and 45 min, respectively. Circular dichroism (CD) spectra of the silk sericins were recorded in water (after dilution) at room temperature in a quartz cell having a path length of 0.10 mm, using a JASCO-20 Recording Spectropolarimeter. CD spectra were also recorded for the *Nd-s* silk sericin thread, *i.e.*, the cocoon silk sericin produced by the larva of the moth, *Nd-s* silkworms, which had been directly spun on the quartz cell. Mole percents of the silk sericin's conformations in the aqueous solution were determined by a linear combination of the three reference conformations, α -helix, β -form and random coil, according

to the method proposed by Greenfield, *et al.*,¹⁰ using the FACOM 230-25 computer.¹¹

RESULTS AND DISCUSSION

The CD spectra of *Nd-s* silk sericin extracted for 5 and 45 min respectively have strong negative bands at 198 nm assigned to the random coil conformation of the silk sericin and weak negative bands at *ca.* 218 nm assigned to the β -structure. The change in the magnitude of these negative CD bands with the extraction time seems to indicate that the random coil fraction decreased and the β -structure fraction increased a little with time. The CD spectrum of *bombyx mori* silk sericin extracted for 5 min was quite similar to those of the *Nd-s* silk sericin, implying that the first extracted fraction of *bombyx mori* took a random coil-rich conformation.

In order to determine the mole percents of the three possible conformations of the silk sericins in the solution phase, calculation was made according to the method proposed by Greenfield, *et al.*¹⁰ Figures 1 and 2 show the observed (solid line) and calculated (broken line) curves for *Nd-s* and *bombyx mori*, respectively. As is seen in Figures 1 and 2, the calculated CD curves approximately agree with the corresponding curves. Particularly good fits were obtained above 210 nm. These results imply that the observed CD spectra of the silk sericins can be adequately approximated by a linear combination of three reference conformations of poly(L-lysine), *i.e.*, α -helix, β -form and random coil. The mole percents of the three conformations in water of these silk sericins extracted are given in Table I. The conformations of *Nd-s* silk sericin extracted for 5 and 45 min were obviously quite similar to each other, the β -form and random coil being 35–36% and 63–64 mol%, respectively. Such negligible helicities of *Nd-s*

Figure 1. Observed (—) and calculated (---) CD spectra of Nd-s silk sericin extracted by water for 45 min.

Figure 2. Observed (—) and calculated (---) CD spectra of *bombyx mori* silk extracted by water for 45 min.

silk sericin as 0.5 and 2.2 mol% within an error of the calculation, may indicate the absence of the α -hlical sequence in the proteins. As is also evident from Table I, the mole percents of the three conformations of *bombyx mori* extracted for 5 min were very similar to that of Nd-s silk sericin, indicating that only the sericin fraction was extracted from *bombyx mori* silk. It should be noted, however, that the β -structure of the *bombyx mori* silk extracted for 45 min was only ca. 23 mol%, the α -helix as high as 10 mol%, and random coil ca. 67 mol% as is shown in Table I.

These results seem to be in a good agreement with the amino acid composition data by Komatsu,¹² that the total amount of the β -structure-forming amino acids, i.e., glycine, L-valine, L-isoleucine, L-cystine, L-serine and L-threonine, decreases from 61.3 to 39.3 mol% with extraction time for *bombyx mori* silk. The total mol% of these β -structure-forming amino acids is higher in the outer core than in the inner core. The α -helix content of 10 mol% for the *bombyx mori* silk extracted for 45 min also coincides with our previous

Table I. Calculated conformations (in mol%) of Nd-s silk and *bombyx mori* silk extracted by water for 5 and 45 min. Calculation was made above 210 nm by a linear combination of the three reference conformations of poly(L-lysine) to get a best fit to the experimental value according to Greenfield, *et al.*¹⁰

	Nd-s silk		<i>Bombyx mori</i> silk	
	5 min	45 min	5 min	45 min
α -Helix	0.5	2.2	1.9	10.0
β -Form	35.6	35.3	34.6	23.2
Random coil	63.9	62.5	63.5	66.8

Figure 3. CD spectra of Nd-s silk sericin thread soon after spinning (a) and after steam treatment (b).

results¹³ in which the silk fibroin began to dissolve in water after 30 min. These results imply that the primary (the amino acid composition) and secondary structures of Nd-s silk sericin are almost the same for all parts of the silk gland, while the *bombyx mori* silk consists of the so-called multi-layered cores.⁴

CD measurement was also made for an Nd-s silk sericin thread. The CD spectrum (Figure 3(a)) of the thread obtained immediately after spinning shows a negative band at 225 nm, which is different from the Nd-s silk in aqueous solution. The CD spectrum of the native Nd-s silk sericin cast onto a quartz disc, though not given in this paper, showed very similar results to that for the Nd-s silk sericin thread. Itoh, *et al.*,¹⁴ reported a CD spectrum of poly(L-glutamic acid) with the β_2 conformation which also gave a negative band at 225 nm. In their β_2 -structure, the glutamic acid side chains are very closely packed

with each other between the β -sheet, as compared with the corresponding β_1 -structure which is formed in an aqueous solution as in the case of the Nd-s silk in the solution. It may be concluded, therefore, that the Nd-s silk sericin thread takes the β_2 -structure similar to the β_2 -structure of poly(L-glutamic acid). When Nd-s silk sericin thread was exposed to steam, (the effect of which has been ascribed to the change in the molecular cohesive forces), a sort of recrystallization may take place in the presence of water.⁹ The CD spectrum (Figure 3(b)) of the steam-treated Nd-s silk sericin thread shows a negative band at *ca.* 225 nm larger than that of the original thread. This implies that the β_2 -structure was enhanced by the steam treatment. This is probably due to a change in the state of the molecular aggregation, *i.e.*, the rearrangement of the molecular chains in the solid state.

REFERENCES

1. O. Kratky, *Monatshefte für Chemie*, **87**, 269 (1956).
2. R. E. Marsh, R. B. Corey, and L. Pauling, *Biochim. Biophys. Acta*, **16**, 1 (1955).
3. Y. Tashiro and E. Otsuki, *J. Cell Biol.*, **46**, 1 (1970).
4. M. Shimizu, T. Fukuda, and J. Kirimura, "Tanpakushitsu Kagaku," vol. 5, S. Akabori and S. Mizushima Ed., Kyoritsu Shuppan Co., Tokyo, 1957, p 317.
5. E. Iizuka, *Biochim. Biophys. Acta*, **181**, 477 (1969).
6. J. Passent, *Biochim. Biophys. Acta*, **147**, 595 (1967).
7. K. Hirabayashi, M. Tsukada, H. Ishikawa, and S. Yasumura, *Sen-i Gakkaishi*, **30**, 459 (1974).
8. K. Kataoka and I. Uematsu, *Kobunshi Ronbunshu*, **34**, 37 (1977).
9. M. Tsukada, *J. Appl. Polym. Sci.*, **22**, 543 (1978).
10. N. Greenfield and G. D. Fasman, *Biochemistry*, **8**, 4108 (1969).
11. T. Kōmoto, Y. Kojima, and T. Kawai, *Makromol. Chem.*, **179**, 1861 (1978).
12. K. Komatsu, *Bull. Seric. Exp. Stat.*, **26**, 135 (1975).
13. M. Tsukada, K. Hirabayashi, T. Kōmoto, and T. Kawai, *Sen-i Gakkaishi*, **34**, T-455 (1978).
14. K. Itoh, B. M. Foxman, and G. D. Fasman, *Biopolymers*, **15**, 419 (1976).