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Pressure- and humidity-induced structural transition of silk fibroin

Kenjiro YAZAWA[1,2*] and Kosuke HIDAKA[1]
[1] Department of Applied Biology, Faculty of Textile Science and Technology, Shinshu University, 3-15-1 Tokida, Ueda 386-8567, Japan
[2] Division of Biological and Medical Fibers, Interdisciplinary Cluster for Cutting Edge Research, Institute for Fiber Engineering, Shinshu University, 3-15-1, Tokida, Ueda City, Nagano 386-8567, Japan

1 Introduction

We investigated the effect of pressure and water molecules on the structural transition of spider and silkworm silk film. The humidity-dependent structural and mechanical properties of silkworm silk film have been reported in previous studies, whereas those of native spider silk film have not been evaluated so far. We prepared the spider and silkworm silk film composed predominantly of amorphous region. The difference in reactivity to pressure and water molecules was investigated between spider and silkworm silk in terms of crystallization behavior. We also evaluated the effect of pressure on crystallization starting from a different conformational situation using hexafluoro-2-propanol (HFIP) that is known to arrange helical structure of spider and silkworm silk fibroin.

2 Experiment

Synchrotron WAXS measurements were performed on the BL10C beamline at Photon Factory, Tsukuba, Japan, using an X-ray energy of 12.4 keV (wavelength: 0.1 nm). The sample-to-detector distance for the WAXS measurements was 258 mm, and the exposure time for each scattering pattern was 10 s. The obtained scattering data were converted into one-dimensional radial integration profiles using the software Fit2D. The data were corrected for the background scattering, and the crystallinity was calculated from the area of the crystal peaks divided by the total area of the crystal peaks and the amorphous halo by fitting the Gaussian function using Igor Pro 8.03 (WaveMetrics, Inc., Portland, OR). The crystallite sizes were evaluated from the (020) peak using Scherrer's equation according to a previous report [1].

3 Results and Discussion

Two-dimensional WAXS data for spider silk films were converted into one-dimensional data by radial integration (Fig. 1). The one-dimensional WAXS plot was then deconvoluted into crystal and amorphous fractions using a Gaussian function. The crystallinity of the spider silk film, which was found to be not dependent on the added pressure, was calculated to be 18.1% ± 3.3% and 18.6% ± 2.5% at 0 MPa and 980 MPa, respectively.

The crystal structure of silkworm silk films was also evaluated by WAXS (Fig. 2). The crystallinity of the silkworm silk films was 8.5% ± 2.3% when incubated at 75 MPa, whereas an increase to 27.6 ± 2.8% was observed when the silk films were incubated at 100 MPa. This increase in crystallinity results from the structural conversion from random coil to β-sheet in the silkworm silk films.

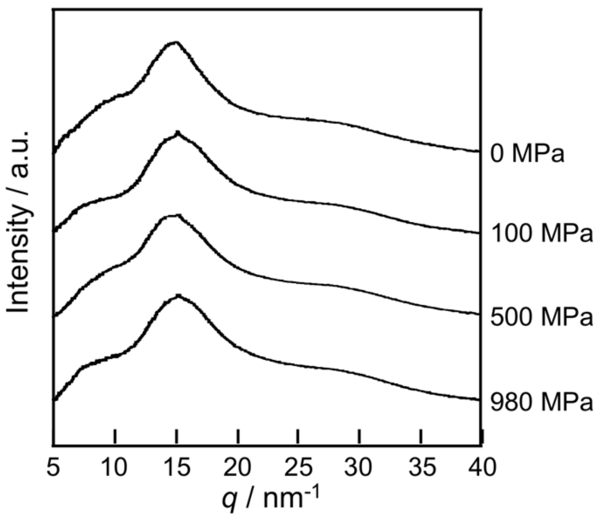

Fig. 1: One-dimensional, radially integrated WAXS profile of spider silk films incubated at different pressure conditions.

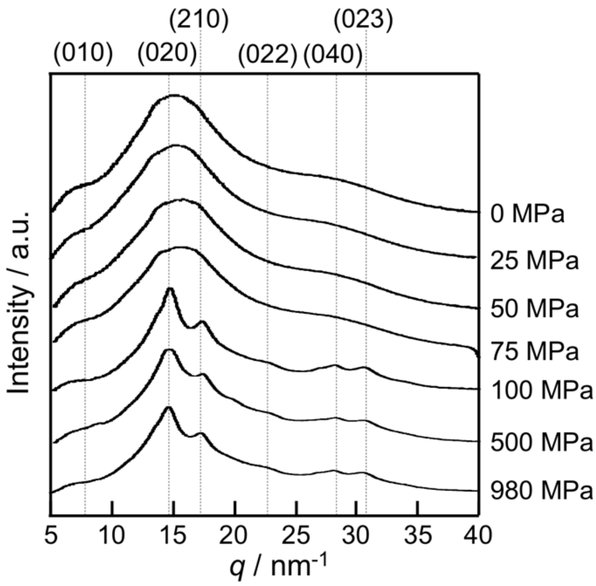

Fig. 2: One-dimensional, radially integrated WAXS profile of LiBr-mediated silkworm silk films incubated at different pressure conditions.

The pressure-induced crystallization of the HFIP-mediated silkworm silk film was also confirmed by WAXS (Fig. 3), and the crystallinity was calculated to be 14.5% ± 1.8% and 28.1% ± 2.8% before and after incubation at 25 MPa, respectively.

It is known that the HFIP-mediated silkworm silk film contains some extent of HFIP, which could not be removed even after the annealing above a boiling point of neat HFIP liquid. Accordingly, there should be a strong interaction between HFIP and silk molecules. The helical structure that forms complex with HFIP molecule facilitated the β-sheet formation of the silk films.

Since the difference between pressure- and humidity-induced crystal structures of spider and silkworm silk fibroin has not been investigated yet, we examined the spider and silkworm silk films incubated at different humidity conditions by WAXS, and compared the results with those of the pressure-dependent analysis. As can be seen in Fig. 4, the β-sheet structure of the spider silk film was induced by incubating at a RH of 97%, although this conversion was not promoted by changing the pressure conditions (Fig. 1). On the basis of the WAXS data, the crystallinity of the spider silk film after incubation at a RH of 97% was calculated to be 23.4% ± 1.1%. On the other hand, the β-sheet of the silkworm silk film was induced by both water (Fig. 4) and pressure (Fig. 2).

The mechanism of pressure- and water-induced crystallization is depicted in Fig. 5. Water-induced crystallization resulted in larger crystallite size but lower crystallinity compared with pressure-induced crystallization. Although pressure did not induce any irreversible structural change of spider silk, humidity facilitated its crystallization. Water molecules are known to interrupt the hydrogen bonds between silk molecular chains and cause plasticization, whereas external pressure

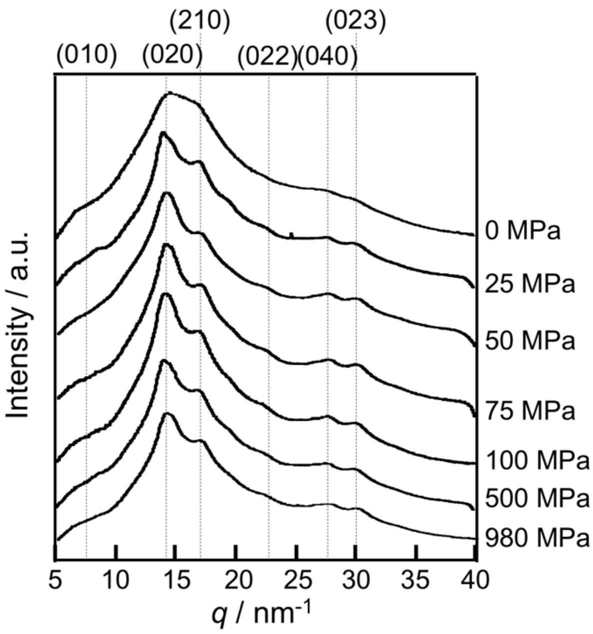

Fig. 3: One-dimensional, radially integrated WAXS profile of HFIP-mediated silkworm silk films incubated at different pressure conditions.

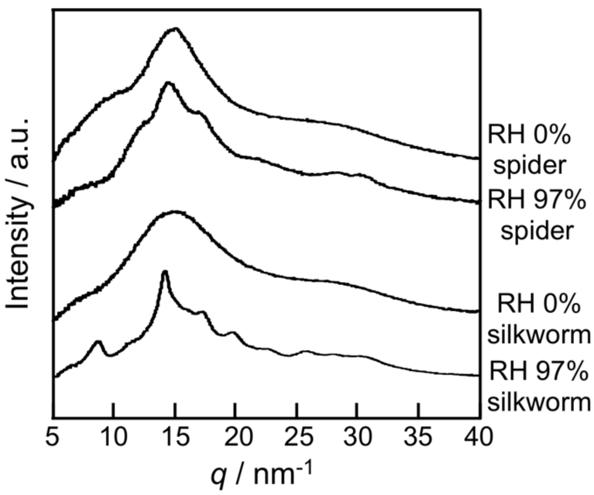

Fig. 4: Comparison of one-dimensional, radially integrated WAXS profiles between spider and silkworm silk films incubated at RH 0% and RH 97%.

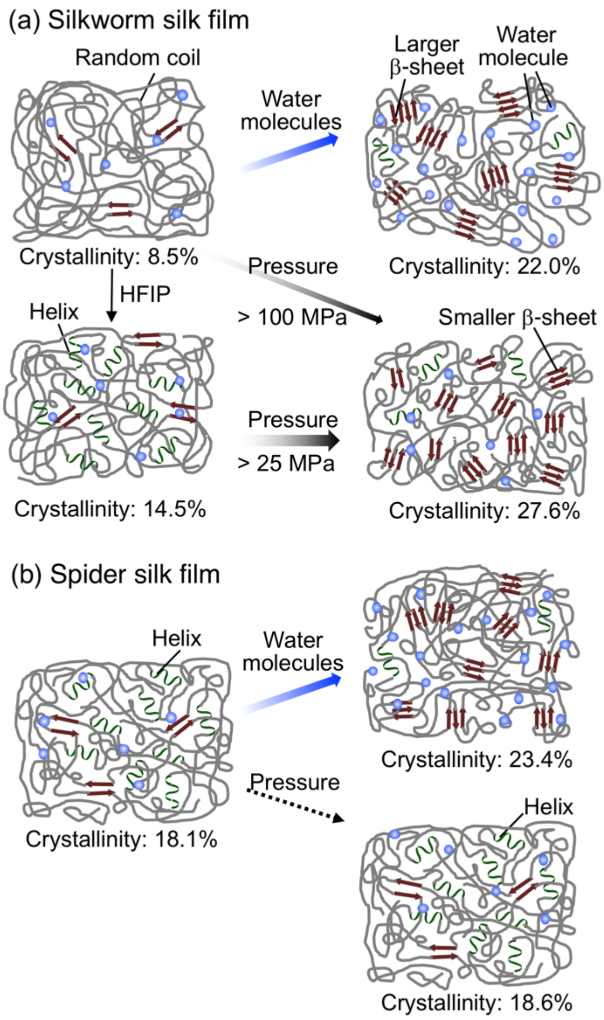

Fig. 5: Schematic illustration of pressure- and humidity-dependent structural transition of spider and silkworm silk films.

forces the local alignment of silk molecular chains. This difference in action between water molecules and pressure might explain the different crystallization behavior of silk fibroin.

Acknowledgement

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References

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* kenjiro_yazawa@shinshu-u.ac.jp