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Combination of X-ray scattering and laser Doppler vibrometry using spider silk reveals a wide frequency range of propagation unlike silkworm silk

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1 Introduction

Both spiders and silkworms produce silk, but the roles of silk fibers are different. Spiders mainly produce silk fibers to build webs, capture prey, and use as a lifeline, while silkworms use silk fibers to protect pupae from predators and maintain the temperature and humidity inside the cocoon.[1] Spider and silkworm silks may differ in vibrational properties not only due to their roles in nature, but also due to the different crystal structures and hierarchical organization.[2] Spider silk is known to have higher degree of molecular orientation along the fiber axis in comparison to silkworm silk.[3] However, the differences in vibrational propagation between spider and silkworm silk at various frequencies have not been investigated so far. In this study, we used combination of X-ray scattering and laser Doppler vibrometer to evaluate sonic properties of spider and silkworm silk. A laser Doppler vibrometer can evaluate the vibrational properties of silk fibers by estimating the velocity of silk fibers based on the difference in frequency of the light that a vibrometer emits and the light that a vibrometer detects when the light reflects from silk fibers (Fig. 1).

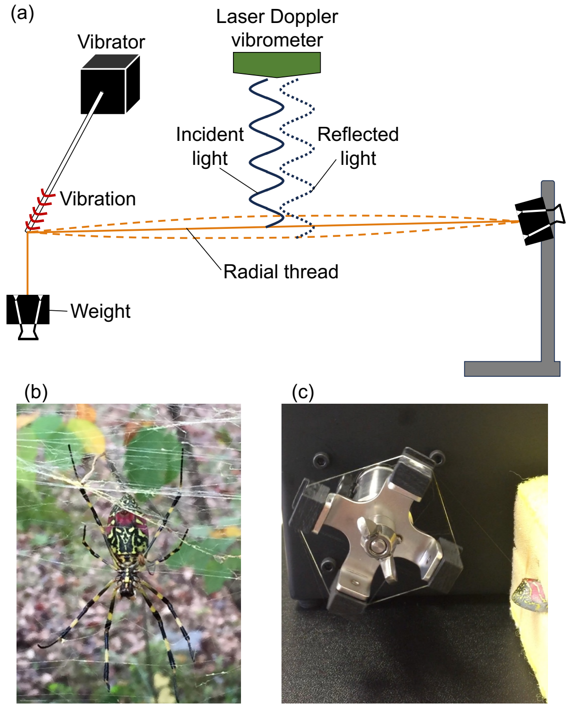


Fig. 1. Evaluation of sonic properties of spider silk using a laser Doppler vibrometer: (a) vibrational analysis performed by the gap of frequency between incident and reflected light derived from the Doppler effect; (b) the abdominal side of a natural spider, *Trichonephila clavata*; (c) collecting spider silk from spinneret of a spider that is fixed between sponges.

2 Experiment

The synchrotron WAXS measurement was performed on bundles of silk fibers using X-ray energy of 12.4 keV (wavelength: 0.1 nm) at the BL-10C beamline of Photon Factory, Tsukuba, Japan. The sample-to-detector distance for the WAXS measurement was 238 mm, and the exposure time for each scattering pattern was 20 s at 25°C under the RH ranging from 30% to 50%. The obtained scattering data were converted into one-dimensional and radially integrated profiles using Fit2D software. The data were corrected for background scattering.

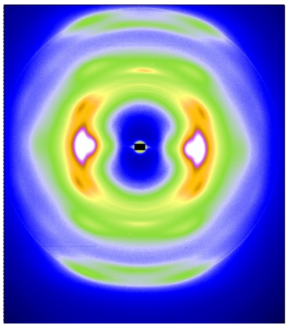
A laser Doppler vibrometer (LV-1800, Ono Sokki Co., Ltd., Japan) was used to examine sonic properties of spider and silkworm silk. The silk bundle was arranged horizontally and then vibrated at the fundamental frequency of the silk bundle (Fig. 1). The fundamental frequency, at which one vibration antinode of the silk bundle was observed, was determined when the center of the silk bundle displayed the greatest amplitude of vibration. Then, the center was focused by a laser, and the gap between the incident and reflected frequencies was used to evaluate the vibrational behavior of the silk bundle. One end of the silk fiber was fixed to the stand with a clamp. The other end was hooked on the vibrating part of the vibrator so that the length from the vibrator to the fixed end was 30 cm. The laser irradiation point of the laser Doppler vibrometer was set 15 cm from the vibrating part. The resultant velocity-time data were averaged using the processing time of 60 s and were converted into velocity-frequency data by fast Fourier transformation (FFT) using FFT analyzer (DS-3000, Ono Sokki Co., Ltd., Japan). The velocity was processed into root mean square value and was subsequently converted into the decibel (dB)-based acoustic level, with 1 m/s being 0 dB. In order to apply tension to the silk bundle, a weight (4.9 g) was hung at the end.

3 Results and Discussion

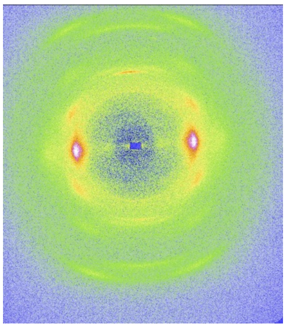
Wide-angle X-ray scattering was measured for three samples: spider silk, silkworm silk, and regenerated silk that was produced by dissolving silkworm silk (Fig. 2). The wide-angle X-ray scattering results for spider silk and silkworm silk presented strong diffraction spots in the equatorial direction, suggesting that the molecular chains consisting of spider and silkworm silks are oriented along the fiber axis. On the other hand, the wide-angle X-ray scattering results for the regenerated silk produced by dissolving silkworm silk showed strong scattering in the meridian direction even after being stretched three times, suggesting that crystals are oriented in various directions other than the fiber axis.

T. clavata spider generally make three-dimensional webs.[4] The shape of a spider web can change depending on the environmental condition.[5] Therefore, it is difficult to estimate the tension of a single fiber based on the spider's weight.[6] It is also unknown the degree of force when the spider pulls the silk with her legs.[7] Based on the average number of radial fibers comprising natural spider webs (approximately 20 fibers) and the weight of adult web-building female spiders (approximately 1.2 g),[8] we applied a 4.9 g weight to the end of the silk bundle consisting of 80 fibers used in this experiment. The resultant decibel-based velocity-frequency profiles are presented in Fig. 3. The fundamental frequency was observed at 191 Hz and 125 Hz for *T. clavata* and *B. mori* silk, respectively. The frequency responses were obtained by exciting the silk bundles at their respective fundamental frequencies. Peaks of decibels were detected at the integer multiples of the fundamental frequency. Spider silk obtained from *T. clavata* maintained peak intensities up to 10,000 Hz (Fig. 3a), suggesting that spider silk can propagate vibrations over a wide range of frequencies. In contrast, silkworm silk demonstrated rapid decibel decrease at higher frequencies (Fig. 3b). Spider silk could propagate vibrations over a wide range of frequencies in comparison to silkworm silk. Because the molecular orientation of spider silk was higher than that of silkworm silk,[9] molecular orientation is most likely crucial for the propagation of vibration.

(a) Spider silk
(*Trichonephila clavata*)



(b) Silkworm silk
(*Bombyx mori*)



(c) Regenerated silk fiber
(Stretching ratio: 3)

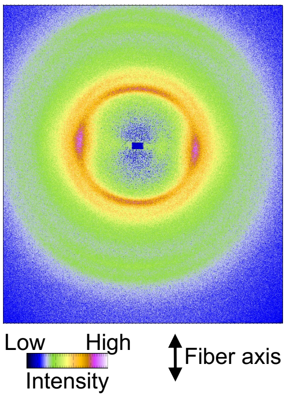
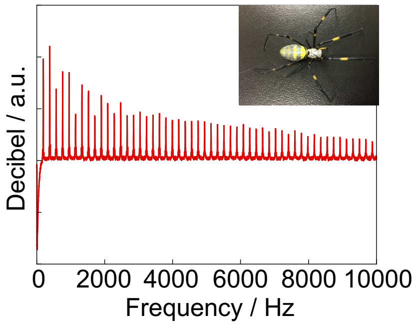


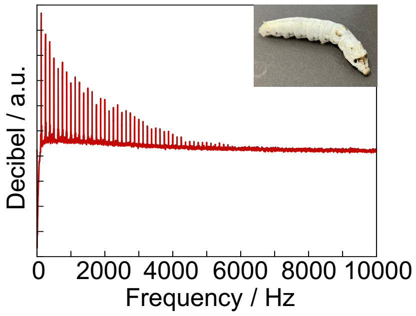
Fig. 2. Wide-angle X-ray scattering of silk samples: (a) spider silk collected from a spider, *Trichonephila clavata*; (b) silkworm silk collected from Bombyx mori; (c) regenerated silk fibers that was stretched at a draw ratio of 3.

Spider silk has a hierarchical structure consisting of a surface skin layer and an internal core layer composed of microfibrils.[10] The skin layer of spider silk can be removed by ether and surfactant treatment and repeated freeze-thaw processes.[11] As a next step, the vibrational properties will be examined when the lipid and skin layers were removed. In addition, we will evaluate the vibrational characteristics of various types of natural spider webs constructed in the field using a vibrator and a portable laser Doppler vibrometer. This work provided a simple detection method to determine how closely the hierarchical structure of the silk being measured compares to that of natural spider or silkworm silk.

(a) Spider silk
(*Trichonephila clavata*)



(b) Silkworm silk
(*Bombyx mori*)



(c) Regenerated silk fiber
(Stretching ratio: 3)

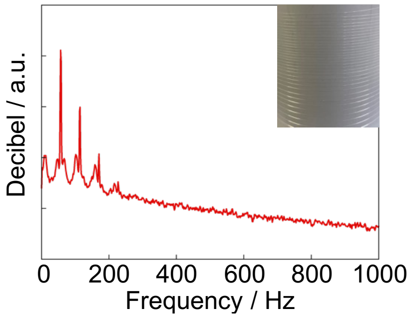


Fig. 3. Evaluation of sonic properties of spider silk using a laser Doppler vibrometer: (a) spider silk collected from a spider, *Trichonephila clavata*; (b) silkworm silk collected from Bombyx mori; (c) regenerated silk fibers that was stretched at a draw ratio of 3.

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