Synchrotron X-ray diffraction of single silk fiber spun by younger instar silkworm larvae

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Introduction
The main component of silk fiber is fibroin, which is a natural fibrous protein synthesized in silk glands of silkworm. Silkworms spin silk fibers in many situations, not only at cocooning. At the exuviation, silkworms spin fibers to fix their bodies, and such fibers are different from ordinary silk fiber. Though silk has been studied for long, the fiber formation process is not clear yet. The differences of silk fibers spun by younger instar silkworm should be attributed to the fiber spinning condition. To study silk fibers spun by younger instar silkworm larvae will provide us with important information on fiber spinning process.

Experiment
Domestic silkworms (Bombyx mori) and wild silkworms (Antheraea pernyi) were bred from the 1st instar larvae to 5th instar larvae, and silk fibers spun at each exuviation was collected. These fibers were treated by 0.5N NaOH to remove sericin, which is another kind of proteins surrounding fibroin fibers. We have also prepared artificially formed fiber samples. 5th instar B. mori was fixed and silk fiber was drawn out from the spinneret at the constant speed of 1, 2, 4 and 8 [cm/s]. These fibers were also treated by NaOH.

The X-ray diffraction experiment was performed at KEK PF-4A using microbeam. The size of X-ray beam was 5µm x 5µm and the wavelength was 1.542 x 10^-10m. The sample fibers were set on the holder at the focus of the microbeam, and cooled CCD camera was located at 15 cm apart from the sample. All the obtained images were background subtracted.

Results and Discussions
For bold fibers (10µm), exposure time was about 100 sec. The younger instar larvae threads are thinner (3 to 5 µm), and longer exposure time was needed (500 to 900 sec.). In the B. mori samples, a strong diffraction spot was observed at 2θ = 19.6° (spacing 4.53Å), and in the A. pernyi samples, spots were at 2θ= 19.5° (spacing 4.55Å) and 2θ= 19.5° (spacing 5.61Å). Each corresponds to 4.3 Å (B. mori) [1], and 4.33, 5.35 Å (A. pernyi) [2] diffractions. Silk thread of grown up larva shows fiber diffraction pattern, but 1st instar thread shows diffraction ring accompanied with fiber pattern. This ring should be attributed to random coil fibroin[3].

Figure 1. Crystallinity of silk fiber (a) dependence on instar of larvae (b) dependence on drawing rate
We have calculated the ratio of crystal part and random coiled part of silk fibers. The calculated crystallinity of silk fiber is shown in the Figure 1. The crystallinity is about 15 to 40%, and increases as the instar of larvae proceeds. On the other hand, the crystallinity does not depend on the drawing rate of the fibers.

Conclusion
Synchrotron X-ray diffraction on single silk fibers shows the co-existence of random coil fibroin and silk-II fibroin. The crystallinity of silk fiber increases with the growth of silkworm larvae. The crystallinity of ordinal silk fiber is about 40%, does not depend on the drawing rate of silk fiber at the spinning.

References

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