SHORT COMMUNICATIONS

Ultraviolet Rays Mechanically Strengthen Spider's Silks

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The resistance of silks to ultraviolet (UV) rays has been one of important characteristics as materials for textiles. It has been shown that UV rays easily decompose the protein molecules of natural silks such as silkworm silks.^{1,2} Therefore, it has been expected that a new silk with the high resistance to UV rays except for silkworm silks should be developed as materials for textiles. And, researchers focused on the mechanical properties of spider silks because the elastic modulus of spider silks is higher than those of amorphous synthetic polymers.^{3–6}

Since a spider captures insects in daytime by using its orb-web which is exposed to sunlight, the spider silks may be affected by the environmental factor of UV rays from sunlight. Thus, it is of great interest to study whether spider silks are mechanically resistant to UV irradiation.

Many properties of spider silks have been studied including mechanical, strain energy, physico-chemical, thermal and optical properties.^{3–16} The chemical degradation of spider's silks was studied by using an electron spin resonance ESR method.^{17,18} To my knowledge, however, no studies concerning the effects of UV rays upon the mechanical properties of spider silks have been reported.

The present study focuses on the effects of UV-irradiation upon the mechanical stress of spider silks and describes findings on the mechanically strengthening of the spider silks ascribed to UV irradiation.

EXPERIMENTAL

Dragline Samples and SEM Observation

Draglines act as a mechanical lifeline for a spider to support its weight.^{19,20} Samples were prepared by cutting long draglines secreted from a *Nephila clavata* spider (Japanese golden-web spider) weighing 540 mg, which was, in September, collected in Matsue, Japan, falling from a wooden bar.^{6,20–22} *N. clavata*

spiders are active in daytime outdoors. Scanning electron microscopy (X-650 SEM, Hitachi, Ltd. Tokyo, Japan) was employed to establish whether the samples were true draglines consisting of double filaments and to determine the cross sectional area of a dragline from the diameter of the double filaments.

Mechanical Measurements

The stress–strain curves for samples with 4 cm long, which were recognized to be true draglines, were carefully measured using a modified TENSILON UTM-IIIIL (Instron Japan Co., Ltd. Tokyo, Japan) with a stretching velocity of 3.3×10^{-4} m/s.^{6,21} A maximum stress at several breaking points observed in the stress–strain curve was defined as the mechanical breaking stress, and the data of the mechanical breaking stress obtained from five samples were averaged. The standard deviation of data was also determined.

Ultraviolet Rays

UV-A, UV-B, and UV-C rays are defined as wavelengths between 320–400 nm, 290–320 nm, and 200–290 nm, respectively. Rays with wavelengths longer than 270 nm and an intensity distribution similar to UV-A and UV-B rays were created by a 660-W xenon-arc lamp (Suntester XF-180, Shimadzu Corp., Kyoto, Japan) and were here designated as UV-C* which contains UV-A, UV-B, and UV-C rays. In the instrument of UV-irradiation, samples were set 25 cm away from the xenon-arc lamp and an air fan was installed in order to avoid heating. The UV-A* and UV-B* used in this study were prepared by excluding wavelengths shorter than 320 nm and 290 nm, respectively, from the xenon-arc lamp using special filters. The UV-B* contains UV-A and UV-B rays while UV-A* contains only UV-A rays. The intensity of UV-B* between 290–320 nm was approximately double the intensity of actual UV-B rays and the intensity of UV-A* between 320–400 nm was

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Figure 1. Stress-strain curves of different sample draglines after the irradiation of UV-A* rays for 0, 5, 14 and 46 h to the draglines secreted from a *Nephila clavata* spider weighing 540 mg. —; before UV-A*-irradiation, — —; 5 h, - - -; 14 h, - · - ·; 46 h.

identical to that of actual UV-A from sunlight. In fact, the intensity of the actual UV-B rays reaching the ground from the sun is very small, while no UV-C rays reach the earth's surface.

RESULTS AND DISCUSSION

Figure 1 shows stress-strain curves of different sample draglines after the irradiation of UV-A* rays for 0, 5, 14 and 46 h to the draglines secreted from a *Nephila clavata* spider weighing 540 mg. The behavior of the stress-strain curves changes with the time of UV-irradiation, giving a remarkable increase in the breaking stress. But, the prolonged exposure of UV-A* irradiation gives a remarkable decrease in the mechanical breaking stress. Here, two kinds of breaking points observed in the stress-strain curve come generally from the breaking of double filaments,²⁰ but more than two breaking points come occasionally from the fibrillation of the filaments due to the prolonged exposure of UV rays. And, a maximum stress at several breaking points observed in the stress-strain curve was used as the mechanical breaking stress.

Figure 2 shows the time dependence of UV-irradiation upon the mechanical breaking stress (BS) of draglines. Here, BS is defined as the mechanical breaking strength divided by the cross sectional area of the draglines. The BS increased rapidly shortly after commencing irradiation with UV-A*, UV-B* or UV-C*, peaked fairly quickly, and then decreased gradually as it approached the respective asymptotic value with time of UV-irradiation. The asymptotic values were lower than that at the starting point of UV-irradiation in all experiments, and were the high-

Figure 2. Time dependencies of the mechanical breaking stress (BS) of draglines (mean value $\pm$ standard error) secreted from a *Nephila clavata* spider weighing 540 mg. Draglines were irradiated with UV rays of UV-A* (>320 nm), UV-B* (>290 nm) or UV-C* (>270 nm), respectively. See EXPERIMENTAL for the definition of UV-A*, UV-B* and UV-C*. The horizontal line is drawn using a value of 1.15 GPa as a starting point. Here, the BS is defined as the mechanical breaking strength divided by the cross section area of the draglines. ●; UV-A*, ○; UV-B*, □; UV-C*.

est for the UV-A*, medium for the UV-B*, and the lowest for the UV-C*. This indicates that the UV-C* strongly destructs the protein molecules of spider silks.

For the BS experiments, the height of the peak was found to increase with increasing wavelength from UV-C* to UV-A*, as shown in Figure 3a. Here, the peak BS was, on average, estimated from the measured data. Compared to the BS at the starting point of UV-irradiation, the peak BS was larger by ca. 50% for UV-A*, ca. 40% for UV-B* and by ca. 8% for UV-C* irradiation. Thus, irradiation with UV-A* showed the highest improvement of BS. The time to reach a peak in BS from the starting point increased with increasing wavelength, and showed a close correlation with the span, which is defined as the time during which BS is higher than that at the starting point of UV irradiation. The span showed an increase with increasing wavelength of UV irradiation with values of ca. 28 h for UV-A*, ca. 7 h for UV-B* and ca. 1 h for UV-C* (Figure 3b). This indicates that UV-A* raises the BS most effectively. Thus, the span is the largest for UV-A*.

In contrast, dragline samples kept in the laboratory with no UV-irradiation, showed no change in BS over a period of three months. This demonstrates that UV-irradiation markedly affects the BS of spider's draglines.

In order to investigate the nature of the occurrence of BS peaks, radicals were measured after UV-irradiation to draglines from *N. clavata* spider. The number

Figure 3. (a) The peak height of BS of draglines secreted from a *Nephila clavata* spider is plotted against the three classes of UV rays; UV-A*, UV-B* and UV-C*. The height is determined from the time dependence of the mechanical breaking stress (BS). (b) The span is plotted against the three classes of UV rays; UV-A*, UV-B* and UV-C*. The span is defined as the time during which the BS is higher than that at the starting point of UV-irradiation.

of radicals ascribed to UV-induced cleavage of chemical bonds²³ increased with increasing the time of UV-irradiation.^{17,18} Radicals give two kinds of factors as follows. The cleavage of the protein molecules due to UV-irradiation¹⁷ may decrease the molecular weight of spider silks and lead to a decrease in the BS, in similar to the case of synthetic polymers.^{24,25} On the other hand, UV-irradiation may induce cross-linking between protein molecules as a consequence of the combination of radicals created by the UV rays. If cross-links are generated, they will increase the molecular weight leading to an increase in the mechanical strength of draglines, in similar to the case of synthetic polymers.^{26,27}

The peaks in the BS shown in Figure 1 may come about as a consequence of two factors; an increase

in molecular weight due to cross-linking of protein molecules and a decrease in molecular weight due to chemical cleavage. This means that the effects of generating cross-links are significant for a period after commencing UV-irradiation, but subsequently the effects of chemical cleavage dominate. The height of the peak may be attributed to the increase in the number of cross-links. Furthermore, the span during which the BS is higher than that at the starting point of UV-irradiation is dependent on the degree of chemical cleavage.

Irradiation with UV-A* took longer to reach the BS peak, the peak was larger and it gave a higher asymptotic value in comparison with UV-B* and UV-C* irradiation. The intensity of UV-B rays reaching the ground is very small, while no UV-C rays reach the earth's surface. Consequently, UV-A may be the most appropriate radiation for strengthening the draglines since the BS maintains a higher level than the initial value for a period of *ca.* 28 h. However, UV-A irradiation of more than 28 h will destroy the functions of the draglines strongly.²⁸

Here we show that especially UV-A rays may effectively contribute to mechanically strengthening the draglines and that *N. clavata* spiders may utilize UV rays to strengthen the orb-web outdoors. In contrast, UV rays did not increase the mechanical strength of silkworm silks or the draglines from *Yaginomia sia* spiders, which are active at night and utilize their orb-web in darkness only. Such a mechanical strengthening of draglines for a *N. clavata* spider which is active on the orb-web in daytime, may be required for keeping the mechanical function of orb-web as long as possible since the UV rays exposed to the orb-web accelerates the chemical cleavage of draglines. On the other hand, the mechanical strengthening of draglines for *Yaginomia sia* spiders, which are active at night, may not be required since no UV rays are exposed to the orb-web.

The mechanical resistance of *N. clavata* spider silks to UV rays may be closely related to the period of rebuilding the orb-web as the adaptation of spiders to the environments of UV rays and it may come from the spider's long history of 400-million-years evolution.

In the future, investigation into strengthening of spider's draglines by UV rays at the molecular level will be necessary to further understand the mechanism of crosslink and then spider silks with the mechanical resistance to UV rays will be useful as a new material for textiles.

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REFERENCES

1. A. Meybeck and J. Meybeck, *J. Photochem. Photobiol.*, **10**, 1 (1969).
2. K. Setoyama, *J. Seric. Sci. Jpn.*, **51**, 271 (1982).
3. J. C. Zemlin, U.S. Army Natick Laboratories, Natic MA, Technical Report TR 69-29-CM (AD 684333), 1968.
4. M. A. Becker, D. V. Mahoney, P. G. Lenher, R. K. Ehy, D. Kaplan, and W. W. Adams, "Silk Polymers," ACS Symposium Series 544, Washington, D.C., 1995, p 185.
5. P. M. Cunniff, S. A. Fossey, M. A. Auerbach, and J. W. Song, "Silk Polymers," ACS Symposium Series 544, Washington, D.C., 1995, p 234.
6. S. Osaki and R. Ishikawa, *Polym. J.*, **34**, 25 (2002).
7. M. W. Denny, *J. Exp. Biol.*, **65**, 483 (1976).
8. R. W. Work and N. S. Morosoff, *Text. Res. J.*, **52**, 349 (1982).
9. J. M. Gosline, M. W. Denny, and M. E. DeMond, *Nature*, **309**, 551 (1984).
10. A. Brandwood, *J. Exp. Biol.*, **116**, 141 (1985).
11. S. Osaki, *Acta Arachnol.*, **37**, 69 (1989).
12. S. Osaki, *Acta Arachnol.*, **38**, 21 (1989).
13. F. Vollrath and D. Edmonds, *Nature*, **340**, 305 (1989).
14. Y. Termonia, *Macromolecules*, **27**, 7378 (1994).
15. A. H. Simmons, C. A. Michal, and L. W. Jelinski, *Science*, **271**, 84 (1996).
16. F. Vollrath and P. H. Knight, *Nature*, **410**, 541 (2001).
17. S. Osaki, *Acta Arachnol.*, **43**, 1 (1994).
18. S. Osaki, K. Yamamoto, A. Kajiwarra, and M. Murata, *Polym. J.*, **36**, 623 (2004).
19. R. F. Foelix, "Biology of Spiders," Harvard University Press, Cambridge, MA, 1982, pp 111–114.
20. S. Osaki, *Nature*, **348**, 419 (1996).
21. S. Osaki, *Int. J. Biol. Macromol.*, **24**, 283 (1999).
22. S. Osaki, *Polym. J.*, **35**, 261 (2003).
23. A. Rauk, D. Yu, J. Taylor, G. V. Shustov, D. A. Block, and D. A. Armstrong, *Biochemistry*, **38**, 9089 (1999).
24. I. Marshall and A. Todd, *Trans. Faraday Soc.*, **49**, 67 (1953).
25. R. Yokota, *Kobunshi*, **39**, 371 (1990).
26. C. J. Grobbelaar, T. A. du Plessis, and F. Marais, *J. Bone Joint Surg.*, **60-B**, 370 (1978).
27. T. Nishikubo, A. Kameyama, K. Tsutsui, and M. Iyo, *J. Polym. Sci., Part A: Polym. Chem.*, **37**, 1805 (1999).
28. S. Osaki, Internationale Fachtagung, Polymerwerkstoffe 2002, Halle, Germany, 2002, pp 617–620.