18. Radiation Degradation of Silk Protein

Wachiraporn PEWLONG[1], Boonya SUDATIS[1],
Hidefumi TAKESHITA[2], Fumio YOSHII[2] and Tamikazu KUME[2]
1. Office of Atomic Energy for Peace, Bangkok, Thailand
2. Takasaki Radiation Chemistry Research Establishment, Japan Atomic Energy Research Institute, 1233 Watanuki, Takasaki, Gunma 370-1292, Japan

Abstract

Silk fibroin fiber from the domesticated silkworm *Bombyx mori* was irradiated using an electron beam accelerator to investigate the application of the radiation degradation technique as a means to solubilize fibroin. The irradiation caused a significant degradation of the fiber. The tensile strength of fibroin fiber irradiated up to 2500 kGy decreased rapidly with increasing dose. The presence of oxygen in the irradiation atmosphere enhanced degradation of the tensile strength. The solubilization of irradiated fibroin fiber was evaluated using the following three kinds of solutions: a calcium chloride solution($CaCl_2/C_2H_5OH/H_2O$ = 1:2:8 in mole ratio), a hydrochloric acid (0.5N) and a distilled water. Dissolution of fibroin fiber into these solutions was significantly enhanced by irradiation. Especially, an appreciable amount of water soluble proteins was extracted by a distilled water.

Key words : Silk, Protein, Fibroin, Radiation Degradation, Dissolution

1. Introduction

The cocoon silk from the domesticated silkworm *Bombyx mori* consists mainly of two proteins; fibroin and sericin. The posterior section of silk glands of silkworm produces fibroin-fibrous, partly crystalline protein, and the middle section produces sericin, which is an amorphous and globulin protein [1]. The fibroin is coated with 20-30% sericin or silk gum. The main amino acid constituents are glycine and alanine, being >70% of the total amino acid content [2 ~ 6]. The compositions of these and other amino acids in the two proteins differ considerably. Recently, studies on silk fibroin have developed remarkably on molecular weight [7 ~ 9] and solubility [8,10]. Most of the previous studies on fibroin have been impeded by the difficulty of applying the usual methods of purification and criteria of homogeneity of protein, which in turn is due to the relative insolubility of the protein [3,11]. However, it is highly sensitive to concentrated alkaline solutions, calcium thioyanate and lithium thiocyanate [2,12,13].

Silk is one of the important resources of Thailand which ranks the seventh largest silk producing country in the world with the production of 1,000 tons per year. More than 10% of silk is discarded each year as silk waste. As silk has excellent physiological characters, it is expected to develop the utilization of silk as new functional biomaterials by the radiation processing technology. A silk fibroin aqueous solution is a starting material for the fabrication of functional biomaterials such as a fine powder for cosmetics and film for enzyme immobilization. Complicated chemical treatments such as solubilization in alkaline or acid solutions followed by neutralization and dialysis are, however, necessary to solubilize fibroin, since silk fibroin is completely insoluble in water. The purpose of the present work is to investigate the application of the radiation degradation technique as a means to solubilize fibroin.

2. Materials and Methods

Silk fibroin fiber, degummed by enzyme, was obtained from Textile Research Institute of Gunma. Chemicals such as calcium chloride($CaCl_2.2H_2O$), ethanol and hydrochloric acid were analytical grade from WAKO chemical company, Japan.

The tensile strength of irradiated silk fibroin fiber was measured using a Sterograph-R1 tension meter (Toyoseiki Co. Ltd., Japan) with a crosshead speed of 50 mm/min. Silk fibroin fiber were irradiated with an electron beam accelerator in the dose range from 500 to 2500 kGy in oxygen atmosphere or vacuum. The electron beam irradiation conditions were 1.0 MV of energy, 5 mA of current and 50 kGy/pass of dose rate.

The solubilization behavior of irradiated silk fibroin fibers was evaluated for the cases of the calcium chloride solution($CaCl_2/C_2H_5OH/H_2O$=1:2:8 in mole ratio)[14], 0.5 N HCl and a distilled water. 50 mg of silk fibroin fiber sample was added into 2 ml of the calcium chloride solution in a glass test tube, heated in water bath at 100°C and then time was measured for the sample to completely dissolve.

For hydrolysis, 10 mg of silk fibroin fiber was added into 5 ml of 0.5 N HCl in a glass test tube. To remove air which dissolve in the solution, the solution was frozen in a liquid nitrogen and remelted gradually under evacuation. Then, the tube was sealed. The sealed tubes were heated at 100°C for 1 h. A dissolved fraction of fibroin was determined by a UV visible spectrophotometer, UV-265 FW (Shimadzu Co. Ltd. Japan) at wavelength of 280 nm. It was found that the optical density at 280nm for irradiated fibroin solutions showed a dose dependence. Thus, optical densities obtained from the above dissolution experiments for irradiated fibroin were normalized by the optical densities for the solution of fibroin irradiated with the same doses and completely dissolved in 1.5 N HCl at 110°C for 18 h.

For dissolution in water, 15 mg of silk fibroin fiber was added into 1.5 ml of a

distilled water in a 2 ml plastic tube and heated at 121°C for 1 h using an autoclave. Any solid residue which did sot dissolve was separated by centrifuging. The separated solution containing dissolved protein was dried by heating at 105°C for 1 h. The residue after drying weighed to calculate the dissolved fraction.

Silk fibroin aqueous solution for an electron beam irradiation was prepared in the following procedure. 250 mg of silk fibroin fiber was added into 50 ml of calcium chloride solution in a glass beaker, and heated in a bath of boiling water until a complete dissolution was attained, generally for 5 to 10 min. Then the solution was dialyzed with a seamless cellulose tube supplied from WAKO. The diameter and length of the tube were 16mm and 15 cm, respectively and 10 ml of the solution was poured into each tube. The fibroin aqueous solution obtained was irradiated with an electron beam accelerator at doses from 50 to 200 kGy. For molecular weight determination, the irradiated silk fibroin were lyophilized and dissolved into a phosphate buffer solution used in GPC(Gel permeation chromatography) analysis.

3. Results and Discussion

Figure 1 showed that the tensile strength of irradiated fibroin fiber decreased with increasing dose. The tensile strength of fibroin fiber irradiated up to 1500 kGy in oxygen decreased to 13% of that for unirradiated fiber, whereas 25% for the same dose in vacuum. The tensile strength for fibers irradiated more than 2000 kGy in oxygen atmosphere could not be measured because of heavy degradation. From the result, it was expected that the higher dose fibroin fibers would be irradiated the higher solubility of the fibers would be attained.

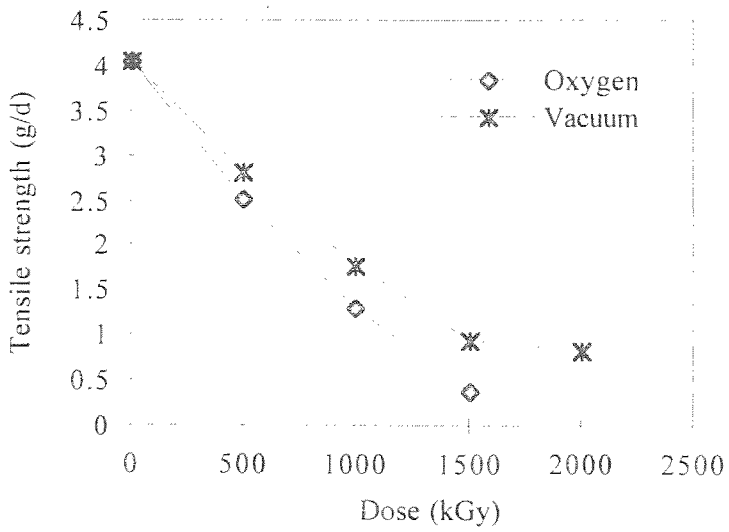

Fig.1 Tensile strength of irradiated silk fibroin fibers

The dissolution behavior of irradiated silk fibroin fiber in the $CaCl_2$ solution was illustrated in Fig.2. The time for silk fibroin fiber to dissolve completely in the solution decreased significantly by irradiation. It is clearly seen from the figure that fibroin fiber irradiated in oxygen atmosphere dissolved in a shorter time compared with that irradiated in vacuum. This trend is consistent with what it was expected from the result of the radiation effect on tensile strength shown in Fig.1.

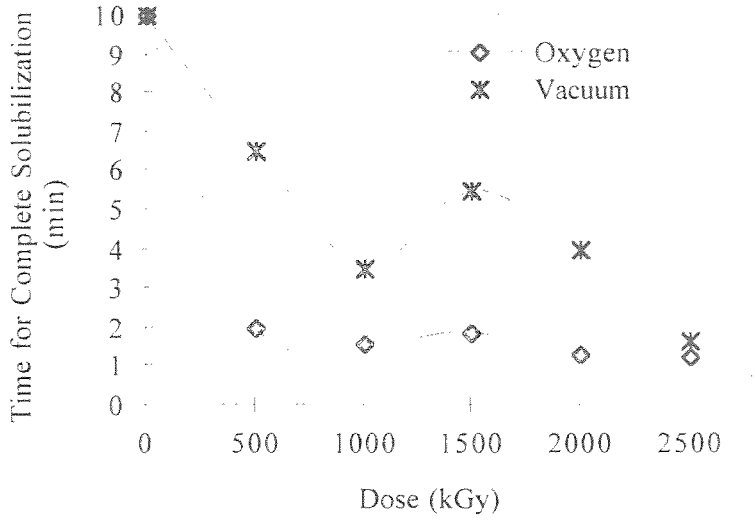

Fig.2 Radiation Effect on dissolution of silk fibroin fibers in the calcium chloride solution($CaCl_2/C_2H_5OH/H_2O$=1:2:8 in mole ratio).

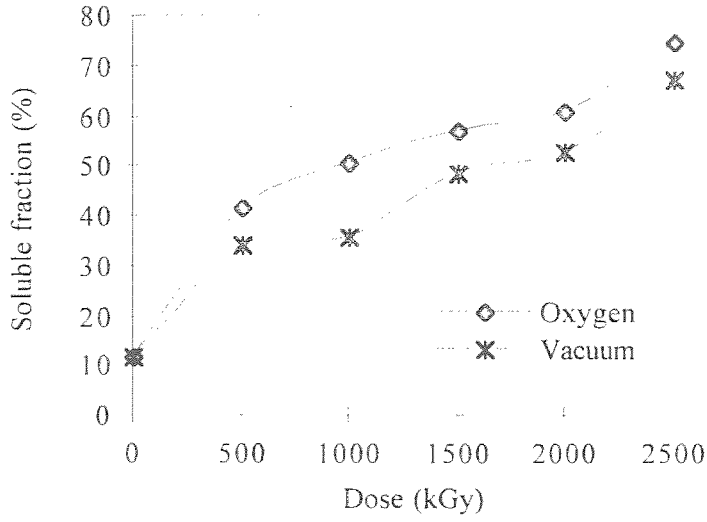

Fig.3 Radiation effect on hydrolysis of silk fibroin fiber in 0.5 N HCl

The dissolved fractions of silk fibroin fiber hydrolyzed in 0.5 N HCl at 100°C for 1 h are summarized in Fig.3. The present dissolution condition is quite mild compared with usual hydrolysis conditions. Generally much stronger dissolution conditions such as 2N HCl at

110°C for 48h or 6N HCl at 110°C for 24 h are used for complete hydrolysis. Only about 10% was solubilized for the unirradiated samples in this experimental condition (Fig.3). Irradiated samples showed higher dissolution. For an example, the samples irradiated up to 2,500 kGy dissolved about 74% for the case in oxygen and about 63% for in vacuum, respectively. It is said from this result that the amount of chemical used in hydrolysis can be greatly reduced even under milder conditions of temperature and reaction time by the radiation degradation treatment.

One of the typical features of silk fibroin fiber is quite insoluble in water. However, it was found that irradiated silk fibroin fiber dissolved in hot water up to 43% depending on dose. The dissolved fractions of silk fibroin fibers irradiated at 2500 kGy were approximately 43% for the oxygen condition and about 30% for vacuum (Fig.4). Although the solubility of irradiated silk fibroin fiber in distilled water was lower than that hydrolyzed in 0.5 N HCl, it should be noticed that no chemical is necessary in this case.

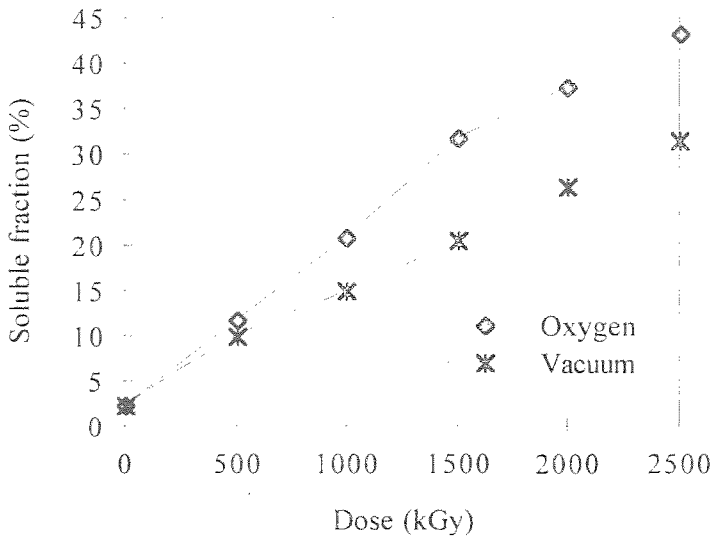

Fig.4 Effect of radiation on solubility of silk fibroin fibers in distilled water

Fig.5 shows GPC curves for fibroin aqueous solution irradiated with an electron beam accelerator. It is obvious that radiation degradation occurred even for irradiation under the solution condition. For irradiated samples, molecular weight decreased with increasing dose. It is also interesting that there seemed to be some discrete pattern in the fragmentation caused by irradiation. This suggests that such discrete pattern reflect the existence of some particular subunits in fibroin molecule which are sensitive to radiation.

4. Conclusion

The application of the radiation degradation technique as a means to solubilize fibroin has been investigated in the present work. It has been demonstrated that irradiated silk fibroin

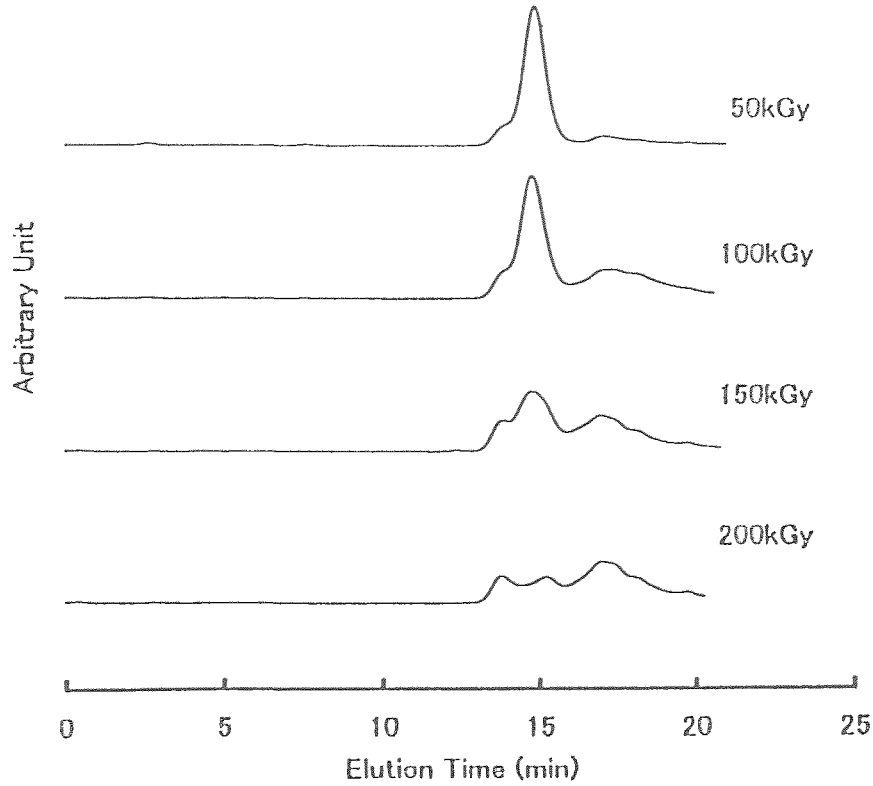

Fig.5 GPC curves for EB irradiated silk fibroin solution. An eluent buffer was 0.1 M sodium phosphate buffer (pH 6.8) + 0.1 M Na_2SO_4

fiber dissolved easily in a calcium chloride solution ($CaCl_2$/ C_2H_5OH/ H_2O = 1:2:8 in mole ratio), a hydrochloric acid (0.5N) and a distilled water. In the case of the calcium solution, dissolution temperatures may be reduced from 100°C down to much lower temperatures for irradiated fibroin fiber. For hydrolysis of fibroin fiber, radiation degradation allows to reduce the amount of chemical used as well as reaction temperature and time. The most interesting point is that radiation degradation of silk fibroin fiber enables to dissolve it into water without any chemical. Of course these fibroin solutions might have a variety of molecular weight depending on the nature of solution and the dissolution condition. It will be a future work to develop applications according to the characteristics of these fibroin solutions.

References

[1] Jadwiga, P., The molecular weight of sericin, *Biophys. Acta.*, **147**, 597-599 (1967).

[2] Kirk, O., Encyclopedia of chemistry technology. Third edition, volume 20, John Wiley & Sons, Inc, (1982).

[3] Sasaki, T., NODA, H., Studies on silk fibroin of *Bombyx mori* directly extracted from silk gland. III. N-terminal analysis and degradation in slightly alkaline solution, *J.*

Biochem., **76**, 493-502 (1974).

[4] Fujiwara, T., Kobayashi, Y., Kyogoku, Y., Confirmation study of ^{13}C-enriched fibroin in the solid state, using the cross polarization nuclear magnetic resonance method, *J. Mol. Biol.*, **187**, 137-140 (1986).

[5] Kato, N., Sato, S., Yamanaka, A., Yamada, H., Fuwa, N., Nomura, M., Silk protein, sericin, inhibit lipid peroxidation and tyrosinase activity, *Biosci. Biotechnol. Biochem*, **62**, 145-147 (1998).

[6] Seifer, S., Gallop, P. M., The proteins (Neurath, H. ed) vol. 4, 201-238, Academic Press Inc. New York.

[7] Sasaki, T., NODA, H., Studies on silk fibroin of *Bombyx mori* directly extracted from the silk gland. II. Effect of reduction of disilfide bonds and subunit structure. *Biochemica. Biophysica. Acta.*, **310**, 91-103 (1973).

[8] Shimura, K., Kikuchi, A., Ohtomo, K., Katagata, Y., Hyodo, A., Studies on silk fibroin of Bombyx mori I. Fractionation of fibroin prepared from the posterior silk gland, *J. Biochem.*, **80**, 693-702 (1976).

[9] Tokutake, S., Okuyama, T., Separation and molecular weight estimate of silk proteins by polyacrylamide gel electrophoresis, *J. Biochem.*, **71**, 737-741 (1972).

[10] Pandit, M.W., Amara, J. S., Narasinga, M. S., Studies on silk fibroin. I molecular weight, sedimentation coefficient, viscosity and optical rotation of silk fibroin from carbonated-extracted silk fiber, *Arch. Biochem. Biophy.*, **149**, 259-268 (1972).

[11] Tokutake, S., Isolation of the smallest component of silk protein, *J. Biochem.*, **187**, 413-417 (1980).

[12] Sridhara, S., Prudhomme, J. C., Daillie, J., Studies on silk fibroin of silkworm *Bombyx mori, Arch. Biochem. Biophy.*, **156**, 168-175 (1973).

[13] Amara, J. S., Narasinga, M. S., Pandit, M. W., Properties of silk proteins extracted in saturated lithium thiocyanate solution, *Indian. J. Biochem. Biophy.*, **15**, 59-61 (1978).

[14] Shaw, J. T., Fractionation of the fibroin of *Bombyx mori* with alkali, *Biochem. J.*, **93**, 54-61 (1964).