Changes in the Fine Structure of Silk Fibroin Fibers Following Gamma Irradiation

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SYNOPSIS

The physicochemical changes of silk fibers irradiated with γ-rays was studied in relation to the amount of absorbed dose in the range 0–21 Mrad. The yellowing index (b/L) suddenly increased at low dose for both raw and degummed silk fibers. An equilibrium value was attained from 10 Mrad upward. The tensile properties were significantly affected by exposure to γ-rays. Both strength and elongation at break decreased at almost the same rate and extent, attaining a final value that was one-half of the untreated control. The birefringence and isotropic refractive index of exposed silk fibers decreased, the effect being larger in the low dose range, suggesting a decrease of crystallinity and molecular orientation. X-ray diffraction curves, however, demonstrated that the crystalline structure remained unchanged even after exposure of the highest γ-ray dose. The thermal behavior evaluated by DSC and TMA measurements showed that the γ-irradiation induced a slight decrease of thermal stability in irradiated silk fibers, this effect being detectable only at 21 Mrad of the absorbed dose. The dynamic viscoelastic behavior suggested that the thermal movement of the fibroin molecules in the amorphous and crystalline regions increased with increasing absorbed dose, attributing to the physicochemical modifications induced by the ionizing radiations.

INTRODUCTION

Developments in the field of nuclear technology have made radiation techniques available to different branches of polymer science. High-energy radiations, such as γ-rays, X-rays, or electron beams, have proved to be a convenient source of free radicals in polymeric matrices for promoting polymerization, cross-linking, grafting, or degradation.[1–3] Moreover, these radiations can be used for an effective sterilization of plastic medical equipment, though the extensive polymer degradation occurring during both exposure and subsequent storage is considered a major practical problem for this process.[4]

A survey of the textile literature published during the last two decades shows that the use of γ radiations in the field of fiber technology has attracted the interest of both scientists and technologists.[5–7] Most papers have dealt with the basic fundamental aspects of the irradiation treatment, i.e., the effects of γ-rays on the physicochemical properties of the irradiated substrates,[8,9] whereas others focused on the development of industrial applications for either synthetic or natural textile fiber processing.[10]

Among the natural fibers, cellulose and keratin fibers are the most extensively studied from the point of view of the γ-ray irradiation treatment. The formation of carboxyl, carbonyl, and aldehyde groups in cellulose has been examined as a function of the total irradiation dose.[11] The cellulose molecules were cleaved at a rate and extent almost proportional to the dosage,[12] and a controlled γ-radiolysis of cellulose for producing microcrystalline cellulose was proposed.[13] Direct dyeings of irradiated cotton fabrics exhibited increased washfastness, though irradiation decreased the dye uptake.[14] Several radiation-grafting processes have already been applied on an industrial scale in the cotton fiber

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sector. Moreover, γ-ray radiation has been suggested as a suitable pretreatment for converting cellulose wastes and lignocellulosic materials to glucose for biotechnological applications.[15-17]

As for the keratin fibers, most studies focused on the modification of wool properties by radiation-induced graft copolymerization of vinyl monomers.[18] An important aspect of the application of inonizing radiations is the damage caused by radiations in the form of partial cleavage of the molecular chains. Depending on the γ-ray dose, cystine bridges and covalent bonds of the protein backbone are broken, as demonstrated by the increase in solubility and by the higher susceptibility of γ-irradiated wool fibers to swelling and disordering agents.[19]

Grafting vinyl monomer onto silk fibers has been considered a powerful method for producing a substantial modification of intrinsic fiber properties, such as crease recovery, photoyellowing, and dyeability.[20] Chemical redox systems in aqueous media have been widely used as an initiator for the graft-copolymerization reaction, and industrial processes were developed on this basis. Photoinitiation systems have been recently been investigated, though their industrial interest is still limited.[21-23]

Radiation-induced graft-copolymerization of vinyl monomers onto silk fibers has received only little attention so far.[24] Since the irradiation with γ-rays may result in chemical degradation and modification of the physical properties of the irradiated substrate, it is of great interest to investigate the effect of this treatment on silk fibers. The present study was undertaken with the aim of elucidating the mechanical, thermal, and structural changes induced by γ-rays onto silk fibers as a function of the irradiation dose.

EXPERIMENTAL

Materials

Silk fiber was obtained after reeling of cocoon threads of the commercial silk worm variety of *Bombyx mori*. The silk fiber was degummed to remove the sericin, which surrounds the silk fiber, in an aqueous solution containing 0.4% soap solution for 2 h at 98–100°C and washed with 0.5% sodium carbonate solution followed by boiling water.

Irradiation

Degummed silk fibers were irradiated at 25°C and a relative humidity (RH) of 65% with γ-rays within the dosage range 0–21 Mrad (absorbed dose). The irradiations were carried out in a γ-cell, at an a 500 Curie ^{60}Co irradiation facility located at the National Institute of Sericultural and Entomoiogical Science, Japan. All experiments were conducted in air. The absorbed dose (Mrad) was estimated from dose of exposure (cpm) using a GM counter, placed at the sample position, considering the periods of exposure time.

Measurements

Yellowness index was obtained using a digital color and difference meter ND-101D (Nihon Denshoku Co.). Physical quality (L/b)[25] was measured by this meter, where L denotes lightness and b denotes yellowness.

The tensile strength and elongation at break of the irradiated silk fibers were measured with a Tensilon UTM-II (Toyo Boldwin Co.) using a standard technique at standard conditions (20°C, 65% RH) at a gauge length of 30 mm, strain rate of 10 mm/min, and chart speed of 200 mm/min.

The differential scanning calorimetry (DSC) measurements were carried out under nitrogen as described in a previous paper.[20] Thermomechanical analysis (TMA) was obtained on a Rigaku Denki instrument at a heating rate of 10°C/min. TMA full-scale and initial force applied to the silk fibers were ±500 μm and 1 gf, respectively.

The dynamic moduli (E'') were measured at 10 Hz with a Toyoseiki Rheolograph Solid-S. The temperature range studied was from room temperature to 270°C and samples were heated at 2°C/min. The sample length was 15 mm, and the initial tension, 30 gf.

X-ray diffraction patterns were obtained using an X-ray source with CuKα radiation ($\lambda = 1.54$ Å). The conditions for the X-ray measurements have been described in detail elsewhere.[25] The refractive indices were measured with the Beche's line method using a polarized microscope under the monochromatic light (Na light) at 20°C and 65% RH.

RESULTS AND DISCUSSION

Yellowness Index

When silk fibers, either raw or degummed, were irradiated, a visible increase in yellowness could be observed. We evaluated, therefore, the rate of whiteness loss by measuring the physical parameter L/b, which expresses the color change[25] in the fibers following γ-irradiations (Fig. 1). The value of b/L for degummed silk fiber irradiated with γ-rays is about two to three times larger than that for the

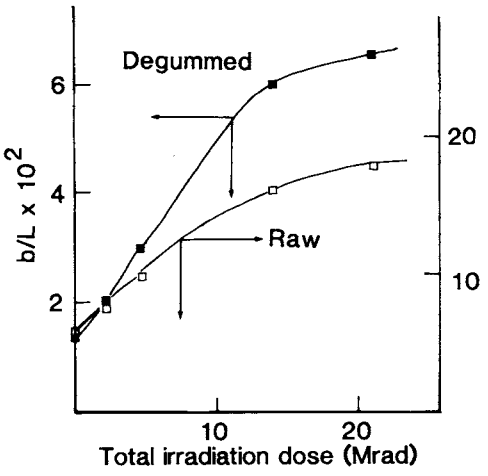

Figure 1 Yellowness index (b/L) of the (■) degummed silk fiber and (□) raw silk fiber following γ irradiation as a function of absorbed dose.

irradiated raw silk fibers with same amount of absorbed dose. The rate of the yellowness increase following γ-ray irradiation was significantly higher for raw silk fibers than for the degummed fiber. From the cross-section view of the bave (raw silk fiber), it is observed that gumming sericin surrounds the silk fibroin fiber (degummed silk fiber). These findings suggest that the exposure to γ-rays onto silk sericin caused an acceleration of the yellowness of the silk fiber. Both of them, however, exhibited a sharp increase of yellowness in the dose range 0–10 Mrad, and then the L/b value seemed to attain a plateau.

It has been reported that the yellowing of protein fibers essentially involves the chemical modification of the side chains of aromatic amino acid residues, such as tyrosine, phenylalanine, and tryptophan. No data are at present available on the effect of γ-rays on the above amino acid residues of silk fibers. However, studying the major changes that occurred in the amino acid composition during γ-radiation of wool, some authors reported that, besides the conversion of cystine to cysteine and cysteic acid, tyrosine and tryptophan residue were extensively degraded.[19]

On the basis of the above findings, it is reasonable to consider that the increase of yellowness of silk fibers following γ-irradiation should be governed by the same basic chemical phenomena, i.e., the degradation of aromatic amino acid residues. The differences observed between raw and degummed silk fibers can be related to their different amino acid compositions.

We would like to draw particular attention to the fact that the degree of yellowness suddenly increased at low γ-ray doses, which permits us to infer that significant chemical changes are induced in the silk fibers at the very first stage of the exposure to ionizing radiations. This is in complete agreement with the results obtained on wool fibers.[26]

Tensile Properties

Tensile strength and elongation at break are very important indications for evaluating the radiation effects on textile fibers. Figure 2 shows the changes in strength and elongation at break of the irradiated silk fibers with γ-rays at different amounts of absorbed dose.

The strength remained almost unchanged as the irradiation dose increased up to about 5 Mrad, then sharply decreased in the range 5–15 Mrad, attaining a value almost constant from 15 Mrad upward. The elongation at break exhibited a rather similar behavior. It is interesting to note that both strength and elongation of the irradiated silk fibroin fiber with the absorbed dose of 21 Mrad attained a final value, which was about one-half that of the untreated control.

Most textile fibers showed a large decrease in breaking stress and extensibility with doses of about 10 Mrad. Delides et al.[12] reported that cotton fibers exposed to ionizing radiation with a dose range similar to that used in the present work exhibited a decrease in tensile properties that could be described by a simple exponential function. The mechanical properties of wool seem to be relatively unaffected by small doses of γ-rays (0.5–10 Mrad). However, Beevers and MacLaren[26] elucidated that this be-

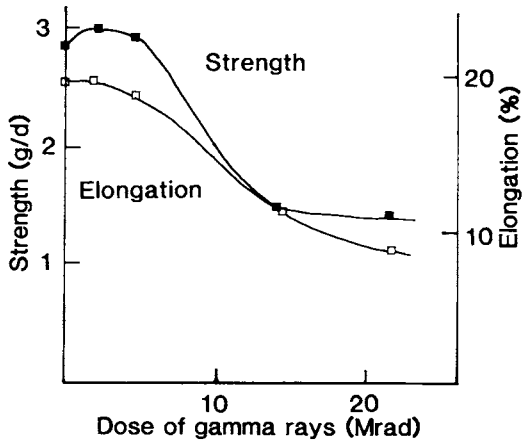

Figure 2 (■) Tensile strength and (□) elongation of the silk fibroin fibers irradiated with γ-rays with different amounts of absorbed dose.

havior was essentially due to the high cross-link density of wool fibers, which made it difficult to detect the changes in physical properties by stress–strain measurements. The apparent initial inertness of wool against irradiation has been explained by the equilibrium between breaking and rebuilding of disulfide cross-links. As the radiation dose was increased over the above range, covalent bond breakage predominated and the changes in the mechanical properties became noticeable.

As is well known, the number of disulfide cross-links in silk fibroin is very small, so that the behavior of silk fibers is comparable with that of other unbranched linear polymers, such as cellulose, whose radiation-induced chain scission becomes apparent from the stress–strain measurements even at relatively small γ-rays doses. The slight increase of tensile strength registered at very small irradiation doses (less than 2–3 Mrad) should be tentatively attributed to the changes induced by γ-rays in the arrangement of hydrogen bridges and polar interactions among the amino acid side chains of adjacent fibroin molecules.

Refractive Indices

The fine structural changes induced in silk fibers following γ-irradiation were studied by refractive index measurements. Table I lists the birefringence and the isotropic refractive index of irradiated silk fibers at different γ-ray doses. Both Δn and n_{iso} values decreased with increasing absorbed dose, the extent of decrease being larger at low absorbed does (0–5 Mrad).

Since birefringence and the isotropic refractive index are measures of the average molecular orientation and crystallinity of silk fibers, the above data imply that γ radiations effected disordering of the fiber structure, which should have involved the arrangement of both the amorphous and crystallinity domains.

These results showed that the effects of γ-rays on silk have been concerned not only with the chemical damage produced by the free-radical formation and main-chain breakage, but also with the apparent modification of the supermolecular organization of the ordered fiber structure.

X-ray Diffraction Curves

X-ray diffraction intensity curves of γ-irradiated silk fibers were examined to ascertain whether some structural changes occurred in the crystalline domains of the fibers irradiated with γ-rays (Fig. 3).

Table I Reflactive Index and Birefringence of Silk Fibers Irradiated with γ-rays with Different Amounts of Absorbed Dose

Sample	Birefringence	n_{iso}
Control	0.056	1.559
Irradiated		
Silk-1	0.055	1.559
Silk-2	0.054	1.558
Silk-3	0.052	1.557
Silk-4	0.050	1.556

Silk-1, -2, -3, and -4 represent the silk fibers irradiated with γ-rays with absorbed doses of 2.2, 4.6, 14.0, and 21.0 Mrad, respectively.

The untreated control (a) exhibited a major X-ray diffraction peak at 20.5°, corresponding to the crystalline spacing [101] of 4.39 Å, which is characteristic of silk fibers with high molecular orientation.

The irradiated silk fibers with absorbed doses of 4.6 and 21 Mrad (b, c) showed X-ray diffraction curves similar to those of the untreated sample. Absence of any shift in the X-ray peak as a result of treatments only implies that the unit cell is not altered and that the major crystalline structure remained unchanged regardless of γ-irradiation.

These data are consistent with those reported for cotton fibers,[15] showing that the crystal lattice structure of cellulose, i.e., elementary cell and crystallite sizes, was almost unaffected even at high γ-ray dose (100 Mrad).

DSC Curves

In consideration of the strong influence of γ-radiation on the physicochemical properties of silk fibers, we studied their thermal behavior by differential scanning calorimetry. Figure 4 shows the DSC curve of the untreated control (a) and γ-irradiated silk fibers with absorbed doses of 4.6 and 21 Mrad (b, c, respectively).

The untreated silk fiber exhibited a major endothermic peak at about 315°C, attributed to the thermal decomposition of silk fibroin with oriented β' configuration.[27] An absorbed dose of 4.6 Mrad did not cause the changes in the thermal behaviors of silk (b), whereas the highest dose of 21 Mrad (c) induced a significant shift to lower value of the decomposition peak temperature (305°C).

The effect of γ-ray irradiation on the thermal behavior of silk fibers is in agreement with the above-reported modifications involving the scission of

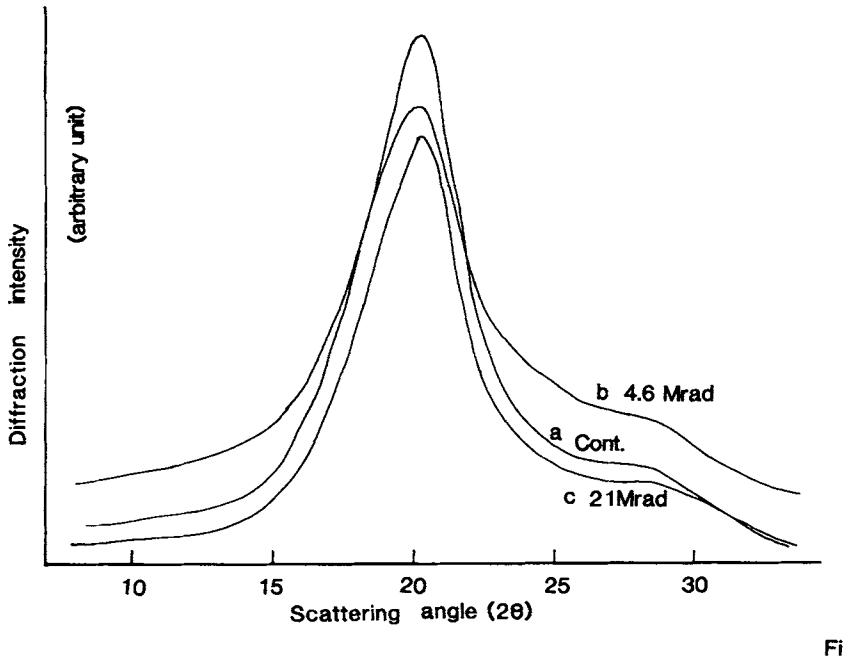

Figure 3 X-ray diffraction intensity curves of the irradiated silk fibroin fibers with different amounts of absorbed dose. Absorbed dose (Mrad): (a) 0; (b) 4.6; (c) 21.

peptide bonds in the amorphous regions and the decrease of molecular orientation.

TMA Curves

The thermal properties of γ-irradiated silk fibers were further investigated by studying the change in the expansion and contraction behavior in the course of the heating process. The TMA curves of untreated (a) and irradiated silk fibers with absorbed doses of 2.2 and 21 Mrad (b, c) are shown in Figure 5.

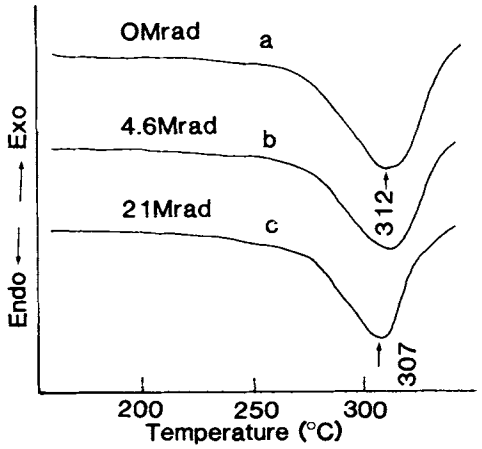

Figure 4 DSC curves of γ-irradiated silk fibroin fibers with different amounts of absorbed dose. Absorbed dose (Mrad): (a) 0; (b) 4.6; (c) 21.

The untreated control (a) heated at a rate of 10°C/min exhibited the typical behavior of silk, with a slight contraction of about 0.7% in the range 25–120°C, followed by a gradual extension, which resulted in the final abrupt elongation under the applied load at above 320°C. The onset temperature

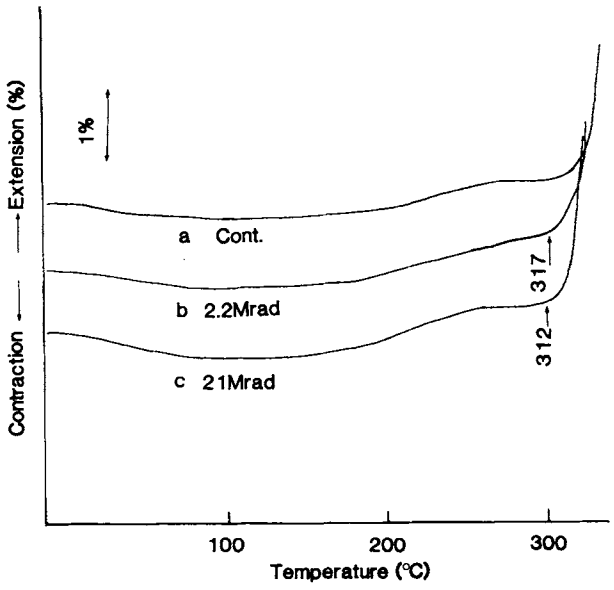

Figure 5 Thermomechanical analysis (TMA) curves of the silk fibroin fibers with different amounts of absorbed dose. Absorbed dose (Mrad): (a) 0; (b) 2.2; (c) 21.

of the final extension shifted to lower temperature for silk fibers irradiated with increasing γ-ray doses (2.2 and 21 Mrad).

It was therefore elucidated that the irradiation influenced the mobility of fibroin molecules and slightly decreased the intrinsic high thermal stability of silk fibers. However, this effect, as well as that previously registered by DSC measurements, became evident only at the upper limit of the irradiation doses tested in the present work (21 Mrad).

Dynamic Mechanical Behavior

Figure 6 shows the temperature dependence of the dynamic loss modulus (E'') of silk fibers irradiated with increasing doses of γ-rays. The untreated control (a) was characterized by a major peak with a maximum at 230°C, whose onset temperature was located at about 180–185°C. This peak has been primarily attributed to the thermal movement of the fibroin chains in the ordered crystalline regions, because the spacing [$d_{(200)}$] corresponding to the intersheet distance[28] was found to gradually expand at above 180°C.

The position of the dynamic loss modulus peak shifted to lower temperature with increasing total absorbed dose (b–d). Accordingly, its onset temperature was found to decrease down to about 150–160°C, especially for the sample irradiated with the maximum dose of 21 Mrad.

The results of the dynamic mechanical behavior imply that the molecular movement of the fibroin molecules was enhanced by γ-irradiation not only in the amorphous, but also in the crystalline regions of silk fibroin. Therefore, the physicochemical modifications induced by γ-rays, i.e., the disordering of the polypeptide chain structure (scission of hydrogen bridges, polar interactions, and covalent bonds) should have also involved the laterally ordered regions located at the end of each crystalline segment, resulting in a different viscoelastic behavior of the β crystal itself in the course of the heating process. X-ray diffraction results, however, demonstrated that the crystalline structure remained unchanged regardless of the irradiation.

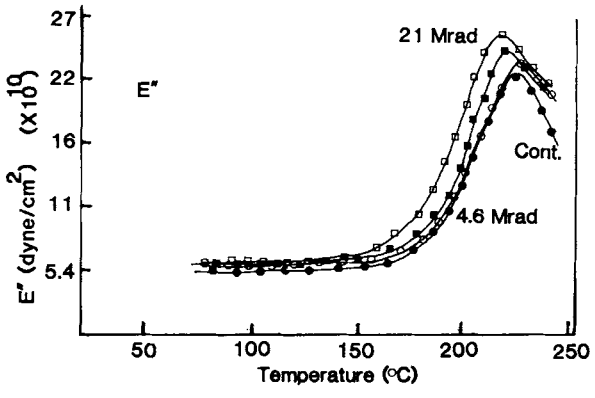

Figure 6 Dynamic loss modulus (E'') of the irradiated silk fibroin fibers with different amounts of absorbed dose. Absorbed dose (Mrad): (●) 0; (○) 4.6; (■) 14; (□) 21.

As observed in the previous section on the basis of the DSC and TMA measurements, significant changes in the thermal behavior of irradiated silk fibers were detected only at high absorbed doses. It has been reported that the maximum radiation dose required for the initiation of the graft-copolymerization of vinyl monomers onto wool fibers is 2 Mrad.[18] In the case of silk, this limit has not been established yet. However, the results obtained in this work show that the thermal and mechanical changes were almost undetectable by the analytical technique adopted at low γ-ray doses, though the occurrence of a certain degree of physicochemical damage induced by the ionizing radiation could not be excluded (see Yellowness and Refractive Index Results).

CONCLUSION

Physical, physicomechanical, and thermal data reported in this paper draw attention to the fact that silk fibers irradiated with γ-rays undergo significant changes in their fine structure and that the extent of modification is related to the absorbed dose. The decrease of average molecular orientation and crystallinity observed by measuring the optical properties, the loss of tensile properties, and the reduced thermal stability of irradiated silk fibers is consistent with the hypothesis that the effect of γ-rays implies the occurrence of several types of physicochemical degradations (free-radical formation, main-chain breakage, disordering of fibroin chains by breaking of hydrogen bonds, etc.). Amorphous and laterally ordered regions are identified as the fiber domains preferentially affected by γ radiations. Crystalline regions seem more stable, since the unit cell is not altered. However, chemical degradation cannot be completely excluded, especially at the highest dose of absorbed radiation. The dramatic decrease of mechanical properties seem to support this hypothesis. From the point of view of industrial application, e.g., for radiation-induced graft copolymerization of vinyl monomers, a treatment not exceeding 5 Mrad of the absorbed dose can be considered sufficiently safe for maintaining almost unchanged or limiting degra-

dation of the physicochemical properties of silk fibers.

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