

Chemical Structure and Dynamic Mechanical Behavior of Silk Fibers Modified with Different Kinds of Epoxides

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SYNOPSIS

The chemical reactivity of epoxide molecules toward silk fibroin was investigated by determining the rate of conversion of reactive amino acid residues. Significant differences were found between two different bifunctional epoxides, diglycidyl ethers of ethylene glycol (E) and resorcinol (R), the former reacting at a higher extent with arginine and tyrosine. The moisture regain decreased by reaction with epoxides, at a variable rate and extent, according to the hydrophobic/hydrophilic properties of epoxides. A two-step behavior was observed when moisture regain values relating to the silk content in modified silk fibers were plotted as a function of the weight gain. Dynamic mechanical data showed that the major loss peak became broader and its temperature shifted to lower values following the increase of weight gain. The loss peak temperatures showed a linear relationship with the amount of weight gain. The fine structural changes induced by reaction with epoxides will be discussed in terms of chemical and steric factors of the epoxides, as well as of epoxide location within the different structural domains of silk fibers. © 1994 John Wiley & Sons, Inc.

INTRODUCTION

Among the chemical modification techniques applied to silks, the reaction with epoxides is one of the most interesting from the point of view of industrial application, since it has proved its effectiveness in improving some inferior properties of silk, such as crease recovery, light resistance, and color fastness to washing.¹⁻⁴ Despite the large number of technological studies and patents covering both conventional (solvent system) and newly developed (pad/batch method) techniques for epoxide treatment,⁵ basic scientific information on this subject is still lacking.

The investigation of chemical, physical, and structural changes induced on silks by various modifying agents, including vinyl monomers, epoxides, and dibasic acid anhydrides, is one of our main in-

terests in the field of polymer and textile science. As concerns epoxide-treated silks, one of the authors has extensively investigated the reactivity of different kinds of mono- and bifunctional epoxides toward silk.⁶ These studies permitted the selection of the most suitable epoxides for silk modification, as well as the setup of the optimum reaction conditions for their application on industrial plants, in order to exploit the advantages of the modification reaction without negatively affecting the intrinsic properties of silk. The effects of epoxides on the mechanical properties related to the hand and texture of modified silks have also been reported.⁷

An extensive study on the fine structural changes induced by reaction with epoxides has recently been undertaken⁶ with the aim of contributing basic knowledge in this field. Molecular orientation and crystallinity of epoxide-treated silks were investigated by measuring their refractive indices and X-ray diffraction patterns. Mechanical and thermal measurements showed that bifunctional epoxides formed cross-links⁶ between adjacent fibroin chains,

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thus increasing their strength and thermal stability. In a study on epoxide-treated tussah silk fibers, we obtained very interesting data on their microstructure by dynamic mechanical analysis.⁸ We found that a linear relationship exists between the amount of weight gain and the dynamic loss peak temperature, which shifted down to lower values as the weight gain increased. Similar results were obtained on *Bombyx mori* silk fibers treated with ethylene glycol diglycidyl ether (E) using the pad/batch method.⁵

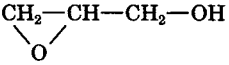
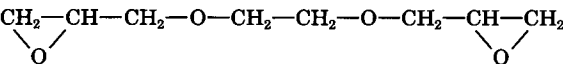
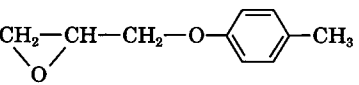
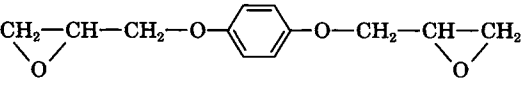
The aim of this article was to further investigate the reactivity of epoxides toward *B. mori* silk fibers, with special attention to two bifunctional epoxides differing in the size and shape of the substituent. Moreover, the equilibrium moisture regain and the dynamic loss modulus curves will be studied as a function of the weight gain. These data, together with those previously reported,⁶ will permit us to widen our knowledge on this modification technique, in order to improve the existing technological applications.

EXPERIMENTAL

Materials

Raw silk fibers were obtained from the reeling of cocoon threads of the domesticated mulberry silkworm, *B. mori*. Two silk threads were twisted together mechanically. Silk fibers were pretreated with an 8.1 g/L NaSCN aqueous solution at 25°C for 5 min and then treated at 68–70°C for different periods of time with different kinds of epoxides according to the method described in a previous article.⁶ The four epoxides used were glycidol (G), ethylene glycol diglycidyl ether (E), tolyl glycidyl ether (T), and resorcinol diglycidyl ether (R) (Table I).

Table I Epoxide Compounds Used in This Experiment

G:	$\text{CH}_2-\text{CH}-\text{CH}_2-\text{OH}$ 
E:	$\text{CH}_2-\text{CH}-\text{CH}_2-\text{O}-\text{CH}_2-\text{CH}_2-\text{O}-\text{CH}_2-\text{CH}-\text{CH}_2$ 
T:	$\text{CH}_2-\text{CH}-\text{CH}_2-\text{O}-\text{C}_6\text{H}_4-\text{CH}_3$ 
R:	$\text{CH}_2-\text{CH}-\text{CH}_2-\text{O}-\text{C}_6\text{H}_2-\text{O}-\text{CH}_2-\text{CH}-\text{CH}_2$ 

Measurements

Dried silk fibers modified with different kinds of epoxide compounds having different values of weight gain were hydrolyzed by heating at 110°C for 20 h in 6N HCl under vacuum. The hydrolyzed samples were filtered through a glass disk to remove small amounts of residues, dried in a rotary evaporator at 40°C, and dissolved in a buffer solution at pH 2.2 (1 mg/mL). The amino acid composition was determined using a Hitachi L-8500-type rapid amino acid analyzer. To analyze the minor and important basic amino acid residues related to the chemical modification with epoxides, a concentrated sample solution (five times higher) was applied for the analyzer.

The dynamic loss moduli E'' were measured at 10 Hz with a Toyoseiki Rheograph Solid-S. The temperature range studied was from room temperature to 250°C and the samples were heated at 2°C/min. The sample length was 15 mm with an initial tension of 30 gf.

RESULTS AND DISCUSSION

Chemical Reactivity

The addition reaction of epoxides to silk fibroin, namely, the amount of weight gain attained, is primarily influenced by the kind of epoxide used and by the reaction system adopted. The epoxide-treated silks used in this study were obtained as described in a previous article,⁶ by reaction in tetrachloroethylene at 70°C for different periods of times (1–3 h). Four kinds of epoxide compounds were used, differing in the number of epoxy groups and in the size of the main chain (Table I). Glycidol (G) and tolyl glycidyl ether (T) are monofunctional epoxides, the latter having the largest tolyl substituent. Ethylene glycol (E) and resorcinol (R) diglycidyl ethers are bifunctional epoxides, the latter having larger molecular weight and size.

The reactivity of epoxides toward functional side groups of silk can be estimated by amino acid analysis. This technique permits us to study the reaction kinetics in terms of addition to the hydroxyl group of tyrosine and to the amine groups of basic amino acid residues. Acidic amino acids, such as aspartic and glutamic acids, react as well. However, the addition to carboxylic groups cannot be estimated because the ester bond is easily hydrolyzed under acidic conditions, such as those used for complete protein hydrolysis. Their study requires the use of a gas chromatography-mass spectrometry system.⁹

The amino acid compositions of silks modified with the bifunctional epoxides E and R are listed in Table II, together with an untreated control sample as reference. G- and T-treated silks were analyzed as well, but the results are not included in Table II because they strictly agree with those already reported by one of the authors, who extensively investigated the reactivity of monofunctional epoxides toward silk fibroin.¹⁻³ E- and R-treated silk fibers with different amounts of weight gain showed a substantial decrease of tyrosine and basic amino acid residues (lysine, histidine, and arginine), following the reaction with epoxides. The other amino acid residues, including aspartic and glutamic acid, remained essentially unchanged or showed only slight changes. Additional peaks appeared in the chromatograms of epoxide-treated silks. These peaks, whose origin can be attributed mainly to tyrosine adducts,³ eluted with a retention time very similar to that of leucine and phenylalanine, therefore interfering with the quantitative evaluation of these amino acids. The reactivity of aliphatic hydroxyl amino acids (serine and threonine) was negligibly small, in good agreement with the results previously reported.⁴

The relation between reaction time and concentration of tyrosine and basic amino acid residues is shown in Figure 1. Lysine and histidine did not show

any difference with respect to the kind of epoxide used, their amount becoming almost undetectable after the reaction of 1 h either in E- or R-treated silks. Both these amino acids are located in the amorphous regions of the silk, which are readily accessible to epoxides. Moreover, the amino and imidazole groups of lysine and histidine, respectively, have always exhibited a great reactivity toward epoxides, irrespective of the kind of epoxide, catalyst, solvent system, and reaction conditions adopted.^{2,4,10} The guanidyl group of arginine showed a very low reactivity during the first hour. Then, as the reaction time increased further, the curve of E-treated silks showed a sharp drop, while that of the R-treated sample continued with the same initial trend, the slope remaining small and constant over the entire range examined. The aromatic hydroxyl group of tyrosine reacted more rapidly with E than with R. In fact, during the first hours, about 60 and 35% of tyrosine residues were converted to epoxide adducts by reaction with E and R, respectively. Then, from 1 h upward, the reaction seemed to proceed with the same rate, as shown by the fact that both E and R curves exhibit almost the same slope. After 3 h, the amounts of tyrosine attained a final concentration of 1.2 mol % for E-treated and 2.3 mol % for R-treated silk fibers. The differences observed between E and R epoxide in the rate and extent of tyrosine

Table II Amino Acid Composition (Mol %) of Silk Fibers Modified with Different Kinds of Epoxide Compounds

	Control	E-9.2 ^a	E-13.0	E-15.2	R-7.8 ^b	R-17.0
Asp	1.41	1.4	1.37	1.3	1.37	1.4
Thr	0.83	0.84	0.81	0.79	0.83	0.82
Ser	10.9	11.16	10.78	10.47	11.13	11
Glu	1.08	1.12	1.1	1.06	1.1	1.12
Gly	45.9	47.82	48.51	48.83	47.19	47.88
Ala	29.2	30.8	31.21	31.52	30.36	30.83
Val	2.2	2.12	2.22	2.26	2.23	2.31
Cys	—	—	—	—	—	—
Met	0.16	0.17	0.19	0.2	0.24	0.19
Ile	0.56	0.57	0.58	0.56	0.6	0.6
Leu	0.45	0.8	0.87	0.87	0.57	0.65
Tyr	5.18	2.02	1.3	1.19	3.37	2.27
Phe	0.6	0.74	0.84	0.75	0.6	0.62
Lys	0.25	0.05	0.03	0.02	0.05	0.04
His	0.14	trace	trace	trace	trace	trace
Arg	0.37	0.36	0.25	0.2	0.35	0.33
Pro	0.64	0.45	0.38	0.46	0.34	0.4
Total	99.87	100.5	100.44	100.48	100.33	100.46

^a Modified silk fiber with ethylene glycol diglycidyl ether with weight gain of 9.2%.

^b Modified silk fiber with resorcinol with weight gain of 7.8%.

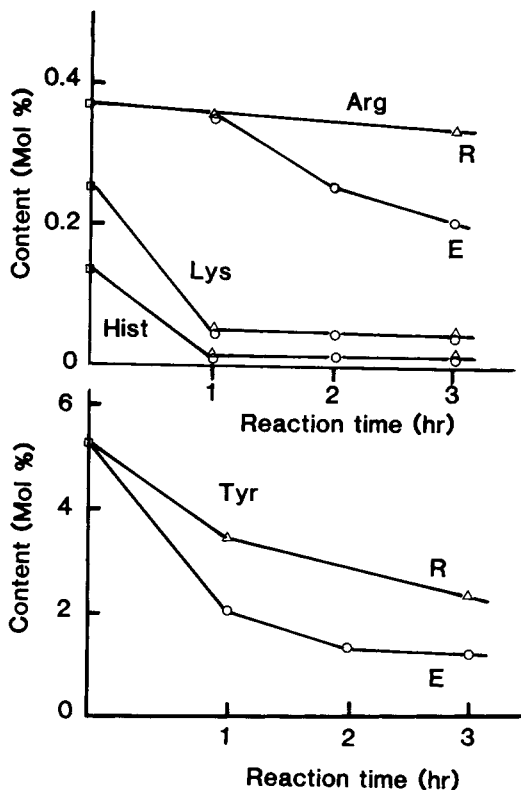


Figure 1 Change in amount of basic amino acids (Arg, His, and Lys) and Tyr residues of the silk fibers modified with ethylene glycol (E) and resorcinol (R) diglycidyl ether as a function of weight gain. Control specimen (□); R-treated sample (Δ); E-treated sample (○).

and arginine conversion can be attributed to both the polar and steric factors characteristic of the epoxide substituents. However, the latter parameter, which refers to the size and shape of the molecule, seems to play the major role in the reaction with silk fibroin.¹⁰

Finally, it is interesting to compare the results of E-treated silks with those recently reported on silks modified with the same type of epoxide by the pad/batch method.⁵ The padding aqueous solution contains the epoxide, together with NaOH and isopropanol. After padding, silks are stored at low temperature (30°C) or less for several hours in order for the addition reaction to proceed. Lysine and histidine residues were found to react almost completely at early stages during storage, confirming their high reactivity toward E, though the reaction conditions were very different. The reaction kinetics of tyrosine showed the same trend as that observed in this article. Its amount decreased more rapidly at the beginning of storage and then attained a final concen-

tration of about 1.6 mol % after 24 h, therefore exhibiting a remarkably high conversion ratio. On the contrary, the concentration of arginine did not show any significant change following the treatment with the pad/batch method, whereas a noticeable degree of conversion was observed by reaction in tetrachloroethylene with NaSCN as the catalyst (see Fig. 1). These results are consistent with the fact that, according to the kind of salt and solvent used and treating temperature, the dissociation constant of the basic guanidyl group can be modified, therefore promoting or reducing the reactivity of ethylene glycol diglycidyl ether (E) toward arginine residues in silks.⁴

Equilibrium Regain

Changes in moisture regain induced on silks by chemical modification with different kinds of epoxides were studied as a function of the weight gain (Fig. 2). The water content is an important physical parameter that, together with other factors, can influence significantly the functional behavior of silks, i.e., comfort, crease recovery, etc.

Two sets of moisture regain values were plotted for each sample in Figure 2. The "observed" values refer to the total weight of modified silks, including silk and epoxides bound to the fibroin chains, whereas the "calculated" values refer only to the amount of silk in the sample. As is well known, epoxides react with polar and ionized groups of various amino acid residues, which are highly effective in promoting the absorption of water by silks. Therefore, from the point of view of the silk substrate, it is interesting to measure the "calculated" values of moisture regain, since they permit evaluation of whether the loss of hydrophilic groups can be compensated by the ability of epoxides to absorb water. The latter feature is clearly related to the chemical properties of the epoxide substituent.

The "observed" moisture regain values of silks treated with different kinds of epoxides decreased gradually over the entire weight gain range examined. Only E-treated silks, whose curve changed from a negative to a positive slope at about 13% weight gain, partly deviated from the above trend. As expected, the decrease of moisture regain was more noticeable for silks modified with the hydrophobic R and T epoxides.

It is now interesting to compare the "calculated" moisture regain curves of silk treated with the bifunctional E and R epoxides. Both curves showed two steps, whose slope and length are clearly related to the properties of each epoxide and to the weight

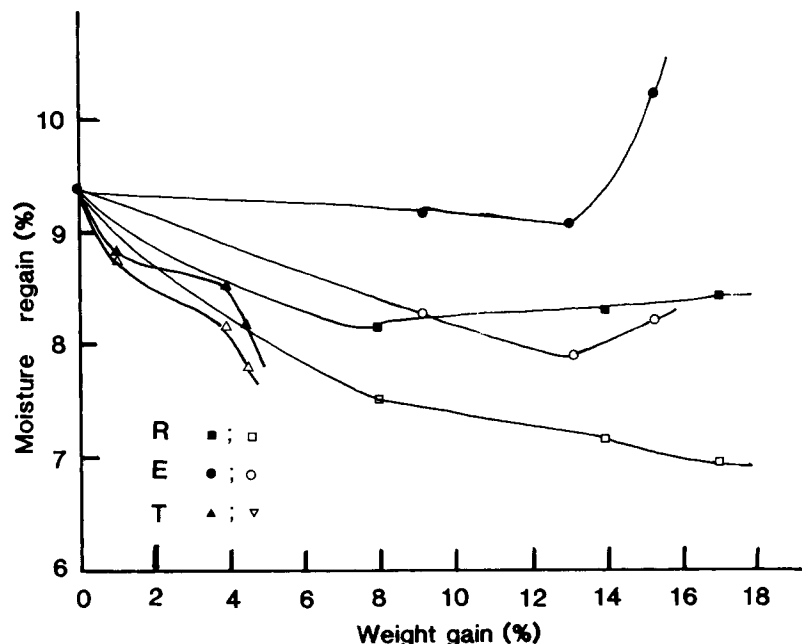


Figure 2 (○, □, △) Observed and (●, ■, ▲) calculated moisture regain of the modified silk fibers with different kinds of epoxides as a function of weight gain. Epoxide: (○, ●) E; (□, ■); R (△, ▲) T.

gain attained. The values of E-treated silks remained similar to that of the control sample until the weight gain attained 13%; then the curve showed a very steep rise in the range 13–15%. The moisture regain of R-treated silks decreased until about 8%, above which value it started to increase, though very slowly and without attaining the level of the reference sample. It has been reported by various authors that cross-links are formed by reaction of silk with bi-functional epoxides.^{4,11} Scientific evidence of this feature have recently been given for both E- and R-treated silks.⁶

On the basis of these findings, we can relate the first step of the moisture regain curves to cross-link formation. During this phase, an important fraction of the hydrophilic amino acid residues are involved in the reaction with epoxides, being therefore excluded from water binding. The net moisture content is determined by the ability of epoxides to absorb water, i.e., by their hydrophobic/hydrophilic properties, as shown by the different behavior of E and R (see Fig. 2). The second step usually takes place after a certain weight gain has been attained and could correspond to the contribution of pendent hydroxyl groups introduced by epoxide. Once again, the rate and extent of moisture regain increase depends on the kind of epoxide used. The small effect observed in the case of R-treated silk fibers can be attributed to the fact that the bulky aromatic ring

present in resorcinol molecules tends to create a hydrophobic environment in its proximity, strong enough to prevent water from binding.

Dynamic Mechanical Behavior

Dynamic mechanical properties are important factors determining the end-use performance characteristics of polymers. As a consequence, the evaluation of these properties is a key point in a variety of fundamental or applied studies. In the present case, it is interesting to verify whether the chemical treatment with epoxides affected the viscoelastic behavior of silk fibers, as well as to determine the kind and extent of the modification, if any, when applied to silk fibers. Dynamic mechanical measurements have been shown to be a sensitive means of detecting both large changes in modulus, such as those associated with the glass-rubber transition and T_g , and secondary transitions and relaxations, which involve relatively small modulus changes and can be attributed to features of the silk fiber's fine structure.¹²⁻¹⁴

The dynamic loss modulus (E'') of untreated silk fibers (curve not shown) remains constant in the temperature range 25–150°C, with only small changes at around 100°C, attributed to segmental motion of fibroin chains in the amorphous regions

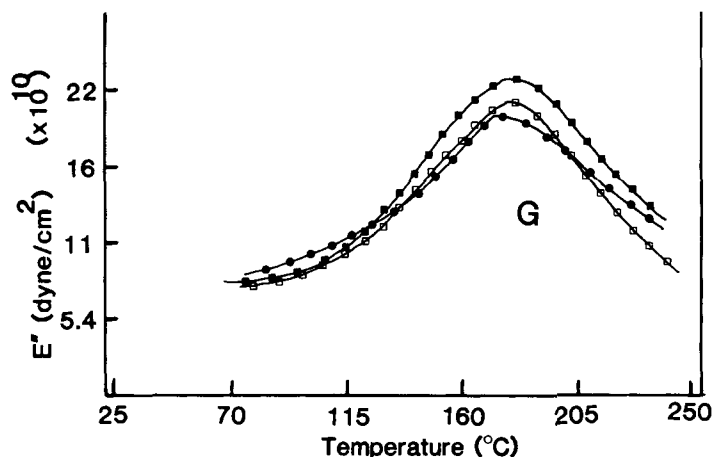


Figure 3 Plots of the dynamic mechanical loss modulus for the silk fibers modified with glycidol (G) with different values of weight gain. Weight gain (%): (■) 0.8; (□) 3.7; (●) 8.0.

following evaporation of water. From about 170°C upward, a large loss peak occurs, with its maximum intensity at 228°C. The onset temperature of the loss peak corresponds to the glass transition temperature of silk fibers,¹⁵ whereas the peak has been attributed to the molecular motion within the crystalline regions, since the spacings [$d_{(002)}$] corresponding to the intersheet distance gradually expand above 190°C.¹⁶

Dynamic loss modulus curves of silk fibers treated with different kinds of epoxides are shown in Figures 3–6. Silk fibers treated with glycidol (G) exhibited a very broad loss peak starting at about 100°C, with a maximum value at 184°C, whose position and intensity did not change with respect to the weight

gain (Fig. 3). These data should be interpreted on the basis of the results reported in a previous article,⁶ where it was found that, under the reaction conditions adopted, G-treated silk fibers underwent an extensive hydrolytic degradation, as confirmed by their very weak mechanical properties. It is therefore uncertain if the low temperature of loss modulus should be attributed to a specific effect of the modifying agent, to the extent of chemical degradation, or to the occurrence of these and other factors influencing the viscoelastic behavior of G-treated silk fibers.

More interesting results emerge from the viscoelastic properties of silk fibers modified with the other epoxides. Toly glycidyl ether (T) did not in-

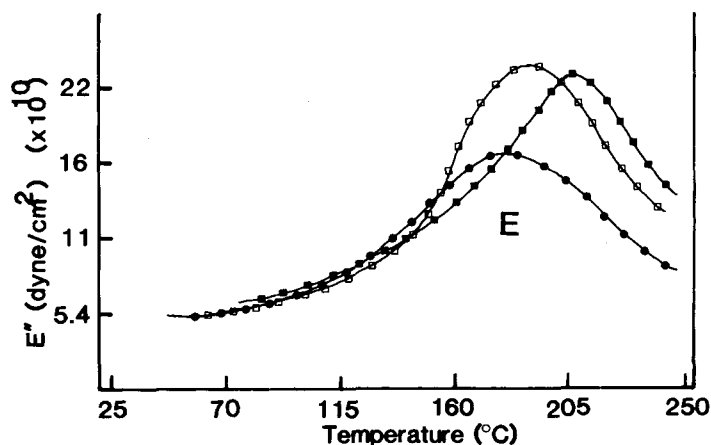


Figure 4 Plots of the dynamic mechanical loss modulus for the silk fibers modified with ethylene glycol diglycidyl ether (E) with different values of weight gain. Weight gain (%): (■) 9.2; (□) 13.2; (●) 15.2.

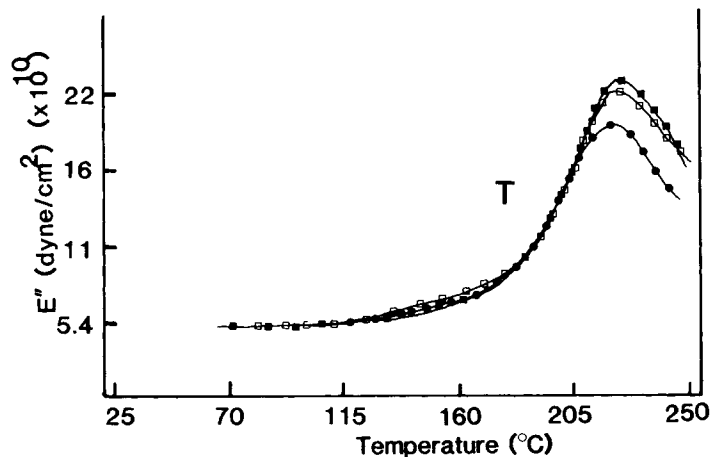


Figure 5 Plots of the dynamic mechanical loss modulus for the silk fibers modified with tolyl glycidyl ether (T) with different values of weight gain. Weight gain (%): (■) 1.0; (□) 3.9; (●) 4.3.

duce significant change in the dynamic loss modulus of modified silk fibers (Fig. 5), with the exception of a slight decrease of peak temperature. The shape of the E'' curve remained essentially unchanged compared with the untreated sample. These findings can be related mainly to the low amount of weight gain attained with the reaction system adopted (1–4.3%). On the contrary, ethylene glycol diglycidyl ether (E) (Fig. 4) and resorcinol (R) (Fig. 6) seemed to exert a larger influence on the viscoelastic behavior of modified silk fibers. The loss modulus peak became broader and its temperature shifted to lower values as the weight gain increased. This behavior could be attributed either to the larger amounts of

weight gain (8–17%) or to a specific effect of these bifunctional epoxides.

To go deeper into this subject, we plotted the values of loss peak temperature as a function of the weight gain (Fig. 7). The most interesting feature is that the values are linearly related to the amount of weight gain, even in the low weight gain range (T). Though the straight lines exhibit different slopes, their intercepts with the ordinate axis fall within the temperature range 225–230°C, where the loss peak temperature of untreated silk fibers is located. A strikingly similar relationship was observed for tussah silk fibers treated with G and E epoxides.⁸

These findings draw our attention to the principal

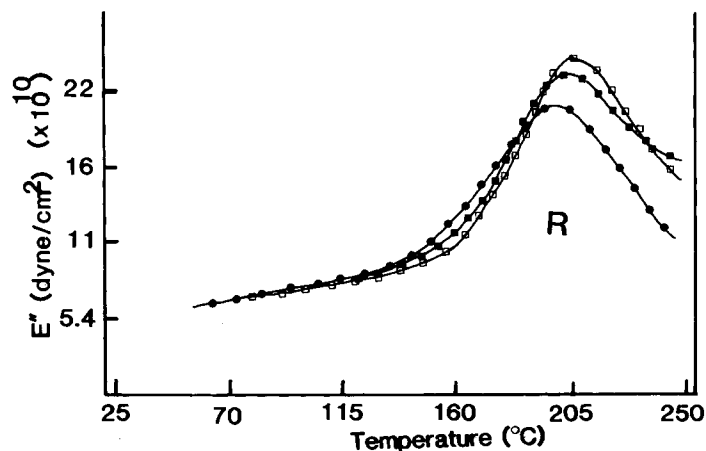


Figure 6 Plots of the dynamic mechanical loss modulus for the silk fibers modified with resorcinol diglycidyl ether (R) with different values of weight gain. Weight gain (%): (■) 7.8; (□) 14.0; (●) 17.0.

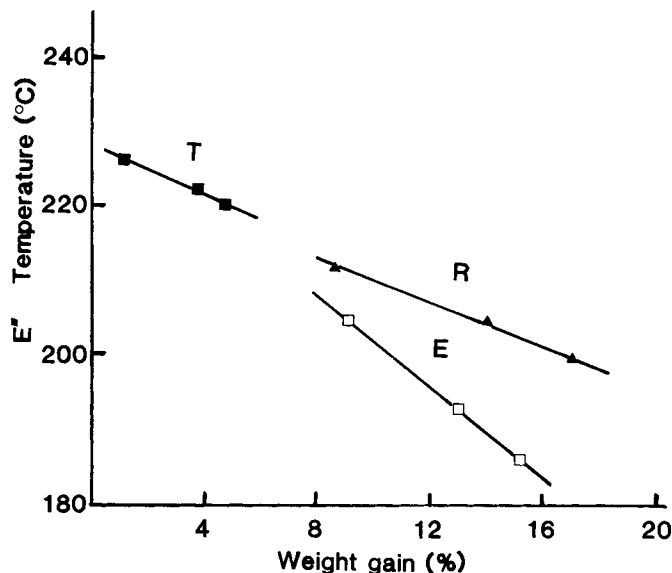


Figure 7 Relationship between the E'' peak temperature and the weight gain of the silk fibers modified with different kinds of epoxides. Epoxide: (■) T; (▲) R; (□) E.

effects induced by epoxide treatment on silk fibers. The molecular motion of fibroin chains in the amorphous regions is noticeably influenced, resulting in lowering the glass transition temperature, as shown by the broadening of loss peak and decreasing of its onset temperature. Moreover, the shift to lower values of the loss peak temperature leads us to suggest that segmental motion within the crystalline regions became less restrictive after reaction with epoxides. The rate and extent of the above phenomena appear clearly related to the amount of weight gain and to the kind of epoxide used. By comparing the two bifunctional epoxides, we can observe that E was much

more effective than was R in modifying the viscoelastic behavior of silk fibers. Taking into account the differences in shape between the two molecules (see Table I) and their different reactivity toward tyrosine (see Fig. 1), we can therefore suggest that epoxide E could bind to reactive sites not only in the amorphous regions, but also in the laterally ordered regions of silk fibers, where tyrosine-rich polypeptide sequences are located.¹⁷

On the contrary, epoxide R was hindered from penetrating the more ordered domains of silk fibers, due to the bulky and rigid benzene substituent. The differences between epoxides E and R are schemat-

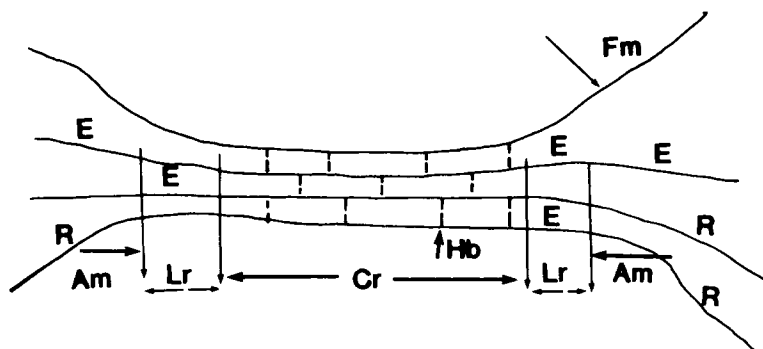


Figure 8 Schematic representation of the location of the attachment of the various kinds of epoxide compounds within silk fiber. A.m: Amorphous region; Hb: hydrogen bonding; Fm: fibroin molecules; Cr: crystalline region; Lr: laterally ordered region; E: ethylene glycol diglycidyl ether; R: resorcinol diglycidyl ether.

ically represented by the model shown in Figure 8, where E molecules are easily immersed in the laterally ordered regions, thus perturbing at a larger extent the viscoelastic behavior of modified silk fibers when subjected to heat treatment.

REFERENCES

1. H. Shiozaki and Y. Tanaka, *Polym. Lett.*, **7**, 325 (1969).
2. H. Shiozaki and Y. Tanaka, *Makromol. Chem.*, **143**, 25 (1971).
3. Y. Tanaka and H. Shiozaki, *Sen-i Gakkaishi*, **45**, 147 (1989).
4. Y. Tanaka and H. Shiozaki, *Makromol. Chem.*, **152**, 217 (1972).
5. M. Tsukada, H. Shiozaki, Y. Goto, and G. Freddi, *J. Appl. Polym. Sci.*, **50**, 1841 (1993).
6. M. Tsukada, M. Nagura, H. Ishikawa, and H. Shiozaki, *J. Appl. Polym. Sci.*, **43**, 643 (1991).
7. M. Tsukada, H. Shiozaki, H. Urashima, and M. Yokozawa, *J. Text. Inst.*, **80**, 547 (1990).
8. M. Tsukada, Y. Gotoh, G. Freddi, M. Matsumura, H. Shiozaki, and H. Ishikawa, *J. Appl. Polym. Sci.*, **44**, 2203 (1992).
9. M. Sakamoto, N. Nohara, M. Wakabayashi, M. Nishimoto, and F. Nakayamai, *Sen-i Gakkaishi*, **40**, T-113 (1984).
10. Y. Tanaka and H. Shiozaki, in *Proc. 7th Int. Wool Text. Res. Conf. Tokyo*, 1985, Vol. 4, p. 441.
11. Y. Kamiishi, Y. Tanaka, and K. Arai, in *Proc. 8th Int. Wool Text. Res. Conf. Christchurch*, 1990, Vol. 1, p. 346.
12. J. Magoshi and Y. Magoshi, *J. Polym. Sci. Polym. Phys. Ed.*, **13**, 1347 (1975).
13. M. Tsukada, H. Shiozaki, J. S. Crighton, and N. Kasai, *J. Appl. Polym. Sci.*, **48**, 113 (1993).
14. M. Tsukada, G. Freddi, P. Monti, A. Bertoluzza, and H. Shiozaki, *J. Appl. Polym. Sci.*, **49**, 1835 (1993).
15. J. Magoshi, Y. Magoshi, S. Nakamura, N. Kasai, and M. Kakudo, *J. Polym. Sci. Phys. Ed.*, **15**, 1675 (1977).
16. M. Nagura, M. Urushidani, H. Shinohara, and H. Ishikawa, *Kobunshi Ronbunshu*, **35**, 81 (1978).
17. M. Tsukada, G. Freddi, M. Nagura, H. Ishikawa, and N. Kasai, *J. Appl. Polym. Sci.*, **46**, 1945 (1992).

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