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**REACTIVITY OF FUNCTIONAL AMINO ACID RESIDUES IN
SILK FIBROIN OF *PHILOSAMIA CYNTHIA RICINI*
TOWARDS MONOEPOXIDES***By Yoshio Tanaka*^{*1}, and *Hideki Shiozaki*^{*2}^{*1} (Research Institute for Polymers and Textiles, 1-1-4 Higashi, Tsukuba, Ibaraki 305)^{*2} (Textile Research Institute of Kanagawa, Aikoh, Kanagawa 243-03, Japan)**ABSTRACT**

The heterogeneous addition reaction of the silk fibroin fibers of *Philosamia cynthia ricini* with various monoepoxides was investigated in ethanol and cyclohexane at 45–75°C after the fibers were padded with aqueous solutions of various salts as catalysts. The reactivity of the main functional amino acid residues was found to decrease in the order: histidine > tyrosine > arginine. The concentration of the aqueous salt solutions influenced the reactions of tyrosine and arginine, but did not affect largely those of histidine and lysine. The reaction rate of tyrosine was discussed in terms of the nucleophilicity of the anions of the catalyst. The reactions of tyrosine and histidine depended on both of the polar and steric factors of the substituents of the epoxides. Their effect was elucidated by the modified Taft equation. The reactivity difference of tyrosine, arginine, and histidine between *Philosamia cynthia ricini* and *Bombyx mori* silk fibroins towards the epoxide was partly explained with swellability and intermicellar distance of the fibroin fibers and the amino acid composition of the fibers.

1. INTRODUCTION

In the salt-catalyzed heterogeneous addition reaction of various monoepoxides with the silk fibroin of *Bombyx mori*, the reactivity of the functional amino acid residues, such as tyrosine, arginine, lysine, and histidine, of the polypeptide chains has been found to depend on the nature of the salt ions, the solvents, and the epoxides, the reactivity of the aliphatic hydroxyl group being negligibly small^{1–6}. Since the *Bombyx mori* silk fibroin contains too small amounts of lysine, histidine, and arginine to permit the reactivity of these amino acid residues to be elucidated kinetically, the epoxide-silk fibroin reaction can be described mainly by the reactivity of the tyrosine residue of the fibroin.

There are similar amounts of tyrosine, and much larger amounts of basic and acidic amino acid residues in the silk fibroin of *Philosamia cynthia ricini* (eri silk) than those in the *Bombyx mori* silk fibroin⁷. The aspect of the

reaction, therefore, may differ between these two silk fibroins. Indeed, the reactivity of the epoxide towards the silk fibroin of *Philosamia cynthia ricini* has been observed to be higher than towards the *Bombyx mori* fibroin^{8,9}. The larger amount of the epoxide addition to the *Philosamia cynthia ricini* silk fibroin seems to be associated with larger intermicellar distance and significant ability of the silk fibroin to swell with aqueous solution¹⁰, besides larger amounts of the reactive amino acid residues, such as tyrosine, histidine, lysine, arginine, and aspartic acid. Thus, the reactivity of these reactive amino acid residues should be different between these two silk fibroins. Consequently, this paper attempts to elucidate the reactivity of these amino acid residues, especially, tyrosine, histidine, and arginine in the *Philosamia cynthia ricini* fibroin towards the monoepoxides in comparison with that of *Bombyx mori* fibroin.

2. EXPERIMENTAL

2.1 Materials

Cocoons of *Philosamia cynthia ricini* were boiled successively with a 2.5% aqueous solution of sodium carbonate for 1 hr and with an aqueous solution containing 2% of Marseilles soap and 1% sodium carbonate for 1 hr, neutralized with diluted aqueous acetic acid solution, and then rinsed several times with warm distilled water. The degummed silk fibroin was extracted first with ethanol, then with ether at room temperature for 24 hr, and finally rinsed with distilled water. Before use the purified, air-dried fibroin was dried in an oven at 105°C for 45 min, placed in a desiccator over silica gel for 30 min., and was weighed accurately.

Epoxides were all of reagent grade and used after distillation under reduced pressure. These epoxides are 1,2-epoxypropane (PO), 1,2-epoxybutane (BO), 2,2-dimethyloxirane (iBO), 1,2-epoxyoctane (OO), 2,3-epoxypropan-1-ol (G), 2,3-epoxypropan-1-amide (GAm), 2,3-epoxypropan-1-al (GAl), phenyloxirane (SO), 1-chloro-2,3-epoxypropane (E), 1-bromo-2,3-epoxypropane (Eb), 1,2-epoxy-3-(2-propenoxy)propane (AGE), 1,2-epoxy-3-methoxypropane (MGE), 1,2-epoxy-3-ethoxypropane (EGE), 1-butoxy-2,3-epoxypropane (BGE), 1,2-epoxy-3-(1-methylethoxy)-propane (iPrGE), 2,3-epoxypropyl acrylate (GA), 2,3-epoxypropyl methacrylate (GMA), 1,2-epoxy-3-phenoxypropane (PGE), 1,2-epoxy-3-(4-methylphenoxy)propane (pTGE), and 1,2-epoxy-3-(2-phenylphenoxy)propane (PPGE). All other chemicals were of reagent grade and were used without further purification.

2.2 Reaction Procedure

A dried and weighed fibroin sample (ca. 0.4g) was impregnated and squeezed with an aqueous salt solution to an approximated 100% wet pickup through a roller mangle. The loosely rolled sample was then placed in a flask containing a 1.43M solution of an epoxide in ethanol or cyclohexane. The flask was joined with a reflux condenser, and held in a thermostatically controlled bath. The reaction was carried out at a desired temperature for various periods with the flask shaken occasionally.

At the end of the reaction, the sample was rinsed with the same kind of solvent as that used in the reaction, followed with acetone, and then extracted with boiling acetone for 30 min, washed with methanol and finally with water. Thus, the remaining epoxide and its oligomer were

removed completely from the sample and could be recovered easily. No oligomers, not bound to fibroin, were detected on further extraction of the fiber with boiling acetone or tetrahydrofuran for several hours. The washed and air-dried samples were dried in an oven at 105°C for 45 min, placed in a desiccator over silica gel for 30 min and weighed accurately. This drying process was sufficient to attain weight constancy for the determination of incorporated epoxides: The extent of the addition of the epoxide was determined from the weight increase of the samples, a correction being made for the weight loss on treatment without epoxides.

2.3 Amino Acid Analysis

Dried fibroin *Philosamia cynthia ricini* (ca. 200mg), untreated or treated with epoxides, was hydrolyzed by heating for 8–16hr at 105–108°C in 20ml of 6N HCl. An undissolved residue of the fibroin (10% or less of total fibroin) was filtered off from the hydrolyzates. The residue was hydrolyzed further and found to consist almost of alanine only by the amino acid analysis.

The amino acid contents were determined with a buffer solution (pH=2.2) of the hydrolyzate (0.3–0.4mg/ml) by use of a JLC-5AH amino acid analyzer (JEOL Ltd). Basic amino acids such as histidine, lysine, or arginine were determined with 3 to 5 fold concentrated solutions of the hydrolyzates (1–2mg/0.8ml). On the hydrolysis of the untreated fibroin for 8, 12 and 16 hr, the contents of the reactive amino acid residues varied as (amino acid contents in mole/10⁵g) Arg., 18.5, 18.5, 18.6; Asp., 40.1, 40.1, 41.0; Glu., 8.0, 8.7, 7.8; His., 16.4, 16.0, 15.9; Lys., 3.1, 3.2, 3.2; Ser., 74.5, 73.4, 73.7; Tyr., 63.7, 64.2, 64.3. Thus, the hydrolysis for 12 hr was adopted to be suitable for determination of these reactive amino acid residues in the *Philosamia cynthia ricini* fibroin as reported previously⁸.

3. RESULTS

The amino acid contents were determined on an amino acid analyzer for the silk fibroin fibers treated with or without various epoxides in different conditions. On treating the silk fibroin with epoxides, the peak areas of tyrosine, arginine and histidine were markedly reduced in the ion exchange chromatograms of the hydrolyzates from those of the untreated fibroin. This implies a loss of these amino acid residues from the silk fibroin and indicates that these residues reacted with the epoxide and

Fig. 1 Automatic ion exchange chromatogram of the hydrolyzates of *Philosamia cynthia ricini* silk fibroins untreated and treated with G, PO, EGE, and BO: I, O-(2,3-dichloropropyl)tyrosine (tentatively estimated but not identified); II, O-(3-chloro-2-hydroxypropyl)tyrosine¹¹; III, O-(2,3-dihydroxypropyl)tyrosine¹¹; IV, O-[2-hydroxy-3-(2,3-dihydroxypropoxy)propyl] tyrosine (tentatively estimated but not identified); V, O-(2-hydroxypropyl)tyrosine¹¹; O-(2-hydroxy-3-ethoxypropyl)tyrosine¹¹; VII, O-(2-hydroxybutyl)tyrosine¹¹.

were modified so that on hydrolysis and elution from an ion exchange column of the analyzer the modified amino acids eluted at different positions from those of unmodified amino acids. On the other hand, the peak areas of aspartic and glutamic acids were unaltered in the chromatograms; this does not imply that these acidic residues were not modified by the treatment with the epoxide. The carboxyl groups of these residues should react with the epoxides to produce the ester groups, which can change into the original amino acids under the hydrolysis condition. Thus, the peak areas did not alter. The modification of these acidic residues, however, can be estimated by the gas chromatographic study¹¹.

Figure 1 shows some ion-exchange chromatograms of the hydrolyzates of the *Philosamia cynthia ricini* silk fibroin untreated and treated with some epoxides. In the chromatogram of the fibroin treated with 1,2-epoxypropane (PO), a decrease in the peak of tyrosine corresponds to the appearance of a new peak V which covers that of phenylalanine. This was found to be due to the formation of O-(2-hydroxypropyl)tyrosine, which was identified by gas chromatography and mass spectrometry¹¹. The silk fibroin treated with 1,2-epoxybutane (BO) has the peak VII corresponding to the O-(2-hydroxybutyl)tyrosine after the peak of phenylalanine¹¹. In the chromatograms of the samples treated with 2,3-epoxypropan-1-ol (G) and 1,2-epoxy-3-ethoxypropane (EGE), the peak (III) of O-(2,3-dihydroxypropyl) tyrosine was observed before tyrosine peak, and the peaks II and VI were identified to correspond to O-(3-chloro-2-hydroxypropyl)tyrosine and O-(2-hydroxy-3-ethoxypropyl)tyrosine, respectively¹¹. Moreover, the peaks I and IV may be due to O-(2,3-dichloro-propyl)tyrosine and O-[2-hydroxy-3-(2,3-dihydroxypropoxy)propyl]tyrosine which were found in the hydrolyzate of the fibroins reacted with G¹¹. This result agrees with that for the hydrolyzates of the *Bombyx mori* fibroins treated with the same epoxides¹²⁻¹⁷.

Gas chromatography-mass spectrometry of the *Bombyx mori* silk fibroin samples treated with various 1,2-epoxyalkanes on an OV-17 column showed¹⁵ that major products were tyrosine adducts, i.e., O-(2-hydroxyalkyl)tyrosines, and that minor ones were serine adducts, i.e., O-(2-hydroxyalkyl)serines. One to one and two to one adducts of the epoxide to lysine were also found to be N^ε-(2-hydroxyalkyl)- and N^ε, N^ε-bis(2-hydroxyalkyl)-

lysines. In the samples¹⁴ treated with 1-aryloxy-2,3-epoxypropanes, O-(3-aryloxy-2-hydroxypropyl)-tyrosines and serines [except for the sample treated with 1,2-epoxy-3-phenoxypropane(PGE)], N^ε-(3-aryloxy-2-hydroxypropyl)- and N^ε, N^ε-bis(3-aryloxy-2-hydroxypropyl)lysines were identified as the main components of adducts. The adducts to glycine and alanine were also detected in the sample treated with 1,2-epoxy-3-(4-methylphenoxy)propane (pTGE), indicating that the amino groups of N-terminal amino acid residues of the *Bombyx mori* silk fibroin react with 1-aryloxy-2,3-epoxypropanes. Finding of 1-aryloxy-2,3-dihydroxy-propanes is considered to indicate that aspartic and glutamic acid residues can react with 1-aryloxy-2,3-epoxypropanes. 3-Aryloxy-2-hydroxypropyl- and bis(3-aryloxy-2-hydroxypropyl)-amines were found in the hydrolyzates, indicating that the arginine and glutamine residues can react with these 1-aryloxy-2,3-epoxypropanes¹⁵. The similar reactions seem to occur with the *Philosamia cynthia ricini* silk fibroin.

The silk fibroin of *Philosamia cynthia ricini* padded with 1 N aqueous solution of KSCN at 100% wet pickup was treated with 1.43M solution of PGE in ethanol at 68°C. As shown in Figure 2, addition of the epoxide increased, and amounts of unreacted tyrosine, arginine, and histidine residues decreased with the time. The amount of lysine residue also decreased with the time, but was too small to be studied kinetically. The consumption rates of these amino acid residues were: histidine > tyrosine > arginine.

Figure 3 shows the effect of the concentration of the catalyst in the padding solution. The sample padded with various concentrations (0-2.0N) of KSCN aqueous solution was treated with 1.43M solution of PGE in ethanol at 75°C for 3hr. Without the catalyst, tyrosine and arginine residues did not react but histidine and lysine residues reacted to 64 and 73% conversions, respectively. A strong effect of the catalyst concentration was found in the reactions of the former residues, while the reactions of the latter ones did not depend markedly on the concentration of the catalyst. This finding is consistent with that with *Bombyx mori* fibroin⁶.

The above variance of the reactivity among these amino acid residues seems to be due to the difference of their dissociation abilities. The pK_i (i = α-COOH, α-NH₂, side group) values of tyrosine, histidine, and arginine

are: 2.20, 9.11, 10.07; 1.82, 9.17, 6.00; 2.17, 9.04, 12.48¹⁸. No change in pH values of the catalyst solution was found after the padding.

In the succeeding experiment, the effect of nature of the catalyst was studied. The silk fibroin fibers were padded with aqueous solutions of a number of sodium salts at constant concentration of 1 N, and then treated with 1.43M PGE solution in cyclohexane at 75°C for 3hr. The reactivities of tyrosine and histidine residues vary with anions of the salts from 1.16 to 54.6 and from 8.85 to 14.8 mole/10⁵g, respectively, as shown in Table 1. The anions of the salts caused enhanced weight gains of the *Philosamia cynthia ricini* fibroin treated with PGE⁸, and the conversions of tyrosine and histidine residues were in the following order of efficacy: CN⁻ > N₃⁻ > S₂O₃²⁻ > SCN⁻ > I⁻ > Br⁻ > CH₃CO₂⁻ > Cl⁻ > SO₄²⁻ > NO₃⁻ > H₂O. This kind of effect has been already reported¹⁻⁶ for the reaction of the silk fibroin of *Bombyx mori* with epoxide in various solvents such as ethanol, cyclohexane, and carbon tetrachloride. Cations of the salts have been also found⁸ to affect the addition of epoxide to the *Philosamia cynthia ricini* fibroin, but no details were studied further in this work.

The effects of the substituents of the epoxide and of the temperature were also investigated. Table 2 shows the reactivity of various epoxides towards the silk fibroin. The fibroin fibers padded with 1 N Na₂S₂O₃ aqueous solution were treated with 1.43M solution of various epoxides in ethanol for 6 hr at different temperatures.

Figure 4 shows some time-conversion curves of tyro-

Fig. 2 Time dependences of addition of PGE and of remaining amounts of tyrosine, arginine, and histidine for the reaction of *Philosamia cynthia ricini* silk fibroin with 1.43 M PGE in ethanol at 68°C, using 1 N KSCN aqueous solution as padding solution of catalyst.

Fig. 3 Effect of KSCN aqueous padding solution on reactivities of tyrosine, arginine, lysine, and histidine for reaction of *Philosamia cynthia ricini* silk fibroin with 1.43 M PGE in ethanol at 75°C for 3 hr.

sine in the reaction with various epoxides such as 1-chloro-2,3-epoxypropane (E), PGE, BO, and G. The samples padded with 1 N aqueous solution of Na₂S₂O₃ at 100% pickup were treated in 1.43M solutions of various epoxides in ethanol at 68°C for 6hr. The reactivity of these epoxides was found in the order: E > PGE > BO > G.

The reactivity of E towards tyrosine in *Philosamia cynthia ricini* fibroin is found to be much higher than that towards the *Bombyx mori* fibroin tyrosine⁶.

Tables 3 and 4 summarize the changes in the conversions of tyrosine and histidine residues in *Philosamia cynthia ricini* fibroin. The order of the reactivity of the epoxides towards histidine residue was as follows: E > 1-bromo-2,3-epoxypropane (Eb) > PO = G > PGE > 1,2-epoxy-3-methoxypropane (MGE) > EGE > 1,2-epoxy-3-(2-propenoxy)propane (AGE) > phenyloxirane (SO) > BO. The epoxides with higher basicity had greater reactivity, except for the epoxides with bulkier substituents, such as PGE, SO, and for the epoxides with smaller substituents like PO.

In the reaction of the tyrosine residues, the higher conversion was found towards PO, E, MGE, EGE, and PGE even at lower temperature than 60°C, while the reaction

with SO and 1-butoxy-2,3-epoxypropane (BGE) was negligible at 75°C. The reactivity order of these epoxides was as follows: MGE > EGE > PGE = E > PO > Eb = AGE > BO > G >> SO at 60°C. This order can not be interpreted by the order of the basicity of the epoxides. The reactivity of tyrosine towards 1-alkoxy-2,3-epoxypropanes was found to decrease with increasing length of hydrocarbon chain in the alkoxy groups.

Tables 3 and 4 also show the temperature dependence

Table 1. Salt effect on the reactivity of tyrosine and histidine for the reaction of *Philosamia cynthia ricini* fibroin with 1,2-epoxy-3-phenoxypropane (PGE)^a

X ^b	Conversion of Tyrosine		Conversion of Histidine	
	mole/10 ⁵ g	% ^c	mole/10 ⁵ g	% ^d
H ₂ O	1.16	1.80	9.67	59.0
NO ₃ ⁻	1.22	1.90	8.85	54.0
SO ₄ ²⁻	1.34	2.08	10.2	62.2
CH ₃ CO ₂ ⁻	5.14	8.00	10.5	64.0
Cl ⁻	3.15	4.90	9.84	60.0
Br ⁻	19.9	30.9	10.3	62.8
N ₃ ⁻	47.6	74.0	13.3	81.1
SCN ⁻	33.4	52.0	11.5	70.1
I ⁻	33.4	52.0	10.4	63.4
S ₂ O ₄ ²⁻	41.8	65.0	11.5	70.1
CN ⁻	54.6	84.9	14.8	90.2

a Silk fibroin padded with 1 N aqueous solution of a sodium salt was treated with 1.43 M PGE solution in cyclohexane at 75°C for 3 hr.

b X is in Na_nX (n = 1 or 2).

c calculated from 64.3 mole/10⁵ g for unreacted tyrosine.

d calculated from 16.4 mole/10⁵ g for unreacted histidine.

Table 2. Temperature and epoxide dependences of epoxide addition (mole/10⁵ g) in reaction of *Philosamia cynthia ricini* fibroin with epoxides^a

Epoxide ^b	Addition of epoxide (mole/10 ⁵ g)				
	45°C	53°C	60°C	68°C	75°C
PO	48	60	73	104	—
BO	17	27	38	60	75 ^c
iBO	12 ^c	15 ^c	20 ^c	26 ^c	31 ^c
E	48	53	55	59	64 ^c
G	44	69	85	128	143 ^c
GAm	132	151	171	—	207
Eb	22	—	32	—	—
SO	10	17	27	42	80 ^c
MGE	89	—	115	—	—
EGE	33	51	93	—	108
AGE	26	38	48	69	92 ^c
PGE	34	59	75	115	122 ^c
pTGE	—	—	85	—	129
PPGE	—	—	—	—	74 ^c
GMA	—	—	—	—	71
BGE	—	—	—	—	20
iPrGE	—	—	—	57	—
GA	—	—	68	—	—
GAl	—	—	—	155	—
OO	—	—	—	—	4

a Silk fibroin padded with 1 N Na₂S₂O₄ aqueous solution was treated with 1.43 M epoxide solution in ethanol for 6 hr at the indicated temperature. Data from refs. 8 and 9.

b The names of epoxides used are shown in the Experimental section of the text.

c Data in this work.

Fig. 4 Time-conversion curves of tyrosine for reaction of *Philosamia cynthia ricini* silk fibroin with various epoxides at 68°C in ethanol, using 1 N $\text{Na}_2\text{S}_2\text{O}_3$ aqueous solution as padding solution of catalyst. R in $\text{OCH}_2\text{CHCH}_2\text{R}$ are: Cl, $\circ$; $\text{C}_6\text{H}_5\text{O}$, $\circ$; CH_3 , $\bullet$; OH, $\circ$.

on these reactions. The reactions of tyrosine residue with E and PGE, and of histidine residue with E, AGE, and G had smaller temperature effect than those with other epoxides under the same condition. The apparent activation parameters were obtained as (epoxide, E_a in Kcal/mole and log A are given): PO, 9.37, 1.36; BO, 11.2, 2.15; E, 1.91, -3.55; G, 9.88, 1.22; SO, 9.17, 0.157; PGE, 3.40, -2.58; AGE, 9.09, 0.798 for tyrosine: PO, 7.26, -0.442; BO, 10.9, 1.58; E, 0.823, -4.60; G, 4.49, -2.28; SO, 11.5, 2.04; PGE, 7.41, -0.376; AGE, 4.25, -2.55 for histidine reaction. These activation parameters are smaller than those for other epoxide reactions¹⁸, but the details are not discussed further in this study, because more precise kinetic analysis is now in progress.

4. DISCUSSION

The kinetic course of the reaction of PGE with tyrosine, histidine and arginine residues is shown in Figure 2, 5 and 6. A sigmoidal curve is obtained by plotting the remaining amount of tyrosine or arginine residues against the reaction time and shows that the reaction has an induction period. The logarithmic relationship between the consumption rate, dw/dt , of each amino acid residues and the remaining amount, $100 - W$, of the residue is shown in Figure 5, where W is the amount consumed given in %. The kinetic order of the arginine or

tyrosine reactions changed with the remaining amount of the residues. The order of the histidine reaction decreased monotonously with the remaining amount of the residue from 3rd to 2nd at the initial stage of the reaction. The consumption rate of tyrosine or arginine residues reaches the stationary stage, when the reaction rate reaches maximum, i.e., the rate being independent of the amount of the residue. The length of the stationary region depends on the nature of the residue.

Figure 6 shows the variations of the rate, dx/dt (mole/ 10^5 g/hr), in ethanol at 68°C with the reaction time (hr). The initial consumption rates, $(dx/dt)_{t=0}$, of tyrosine and histidine residues were obtained as 6.5 and 15×10^{-5} mole/g/hr, respectively, by extrapolating the rate to time zero. The maximum consumption rates, $(dx/dt)_{\text{max}}$, of arginine and tyrosine groups were also determined as 3.7 and 12×10^{-5} mole/g/hr, respectively. These values for the reaction rate of the reactive residues in *Philosamia cynthia ricini* fibroin are similar to those in *Bombyx mori*: The initial reaction rate of tyrosine, and the maximum rate of arginine and tyrosine residues in *Bombyx mori* fibroin were found⁶ to be as 6.2, 1.3 and 13.5×10^{-5} mole/g/hr in ethanol at 68°C, respectively. The kinetic course of the reaction shows, moreover, that the histidine reaction should not be so complicated as that of tyrosine or arginine groups. More detailed analysis of the kinetic course is now in progress and will be reported elsewhere.

Figure 7 shows the dependence of the reactivities of tyrosine and histidine residues on the Edwards nucleophilic parameter, En^{20} , of anions, X^{-n} , of the catalysts, Na_nX . In the reactivity of tyrosine, the points for the anions with larger basicity, Hn , such as N_3^- , were found to deviate from the linear relationship. The reactivity of histidine residue did not depend largely on the En value, suggesting that the active species are dissociated imidazole ions rather than associated ion pairs or complex in the reaction of histidine with the epoxide in the experimental condition. If the effect of the anion basicity, Hn , is negligibly small, the following equation is obtained:

$$\log(W - W_0) = a \text{En} \quad (1)$$

For the reactions of tyrosine and histidine of *Philosamia cynthia ricini* fibroin, a -values were obtained as 0.715 ($r = 0.914$) and 0.0533 ($r = 0.743$), respectively. The value for tyrosine reaction is larger than that [$a = 0.506$ ($r =$

Table 3. Temperature and epoxide dependences of reactivities of tyrosine in reaction of *Philosamia cynthia ricini* fibroin with epoxides^a

Epoxide ^c	Conversion of tyrosine ^b							
	45°C		53°C		60°C		68°C	
	I	%	I	%	I	%	I	%
PO	17.2	26.7	28.3	44.0	33.3	51.8	—	—
BO	5.92	9.21	10.7	16.6	15.1	23.3	28.0	43.5
E	28.7	44.6	32.8	51.0	35.2	54.8	42.5	66.1
G	5.34	8.30	8.68	13.5	13.5	21.0	20.6	32.1
SO	1.22	1.90	2.51	3.90	3.99	6.21	4.44	6.91
PGE	24.6	38.2	31.5	49.0	35.6	55.4	39.9	62.0
AGE	6.43	10.0	11.6	18.0	19.6	30.5	22.5	35.0
MGE	—	—	—	—	46.7	72.6	—	—
EGE	—	—	—	—	41.6	64.7	48.5 ^d	75.5 ^d
Eb	—	—	—	—	19.7	30.7	—	—
BGE	—	—	—	—	—	—	5.52 ^d	8.6 ^d
iPrGE	—	—	—	—	—	—	22.4 ^d	34.9 ^d
pTGE	—	—	—	—	—	—	54.4 ^d	84.6 ^d
GMA	—	—	—	—	—	—	49.2 ^d	76.5 ^d
GAm	—	—	—	—	—	—	61.0 ^d	94.8 ^d

a Silk fibroin padded with 1 N Na₂S₂O₃ aqueous solution was treated with 1.43 M epoxide solution in ethanol for 6 hr at the indicated temperature.

b I, conversion in mole/10⁵ g; %, calculated from 64.3 mole/10⁵ g for unreacted tyrosine.

c The names of epoxides used are shown in the Experimental section of the text.

d obtained at 75°C.

0.847)] for *Bombyx mori* fibroin tyrosine. The dispersion observed in this relationship suggests that another factor or Hn plays a part in the reaction catalyzed by the anions. A detailed study is now in progress and will be published elsewhere.

In the homogenous reaction of various nucleophilic species with E and G in solution, the nucleophilic dependence, a , was found to be 2.46 and 2.52, respectively²⁰. Thus, the effect of the anions, X⁻ⁿ, on this reaction should be as small as 1/4 to 1/3 of those on the above reactions.

If the transition-state species for the reaction between an epoxide and functional groups of amino acid residues are of type Ia or Ic, the reaction should depend on the acidity of cations, M⁺ⁿ. The addition of the epoxide to

the silk fibroin of *Philosamia cynthia ricini*, indeed, has been found to depend on the acidity of the cations⁸. Basicity may play an important part for the transition-state species Ib, while the nucleophilic reactivity towards carbon atom, such as described by Edwards, En²⁰ and the Swain-Scott, Sn²¹, must play a part for an activated complex like Id, because the steric constraint in Id should be greater than in the corresponding ion pair type Ib.

In the homogeneous reaction of epoxides in solution, reaction through transition states of type Ic and Id has been found¹⁹ to be affected by the counterions such as

Table 4. Temperature and epoxide dependences of reactivity of histidine in reaction of *Philosamia cynthia ricini* fibroin with epoxide^a

Epoxide ^b	Conversion of histidine					
	45°C		53°C		60°C	
	mole/10 ⁵ g	% ^c	mole/10 ⁵ g	% ^c	mole/10 ⁵ g	% ^c
PO	7.94	48.4	10.9	66.5	13.3	81.1
BO	2.69	16.4	3.69	22.5	5.97	36.4
E	14.7	89.6	15.0	91.5	15.6	95.1
G	9.17	55.9	11.4	69.5	12.6	76.8
SO	3.13	19.1	5.02	30.6	7.07	43.1
PGE	7.31	44.6	9.76	59.5	12.4	75.6
AGE	7.35	44.8	8.81	53.7	9.94	60.6
Eb	—	—	—	—	15.0	91.5
MGE	—	—	—	—	10.4	63.4
EGE	—	—	—	—	10.1	61.6

a Silk fibroin padded with 1 N Na₂S₂O₃ aqueous solution was treated with 1.43 M epoxide solution in ethanol for 6 hr at the indicated temperature.

b The names of epoxides used are shown in the Experimental section of the text.

c Calculated from 16.4 mole/10⁵ g for unreacted histidine.

Fig. 5 Dependences of conversion rate, dw/dt , of tyrosine, arginine, and histidine on their remaining amounts, $1 - W$, for the reaction of *Philosamia cynthia ricini* silk fibroin with 1.43 M PGE in ethanol at 68°C, using 1 N KSCN aqueous solution as padding solution of catalyst.

Fig. 6 Time dependences of conversion rate, dw/dt , of tyrosine, arginine, and histidine for reaction of *Philosamia cynthia ricini* silk fibroin with 1.43 M PGE in ethanol at 68°C, using 1 N KSCN aqueous solution as padding solution of catalyst.

M^+ or X^- more than that through activated complexes of type Ia or Ib. Therefore, the reaction of dissociable histidine groups under the experimental conditions should not be affected by ions so much as the reactions of the epoxide with tyrosine and arginine residues.

Fig. 7 Effect of nucleophilic parameter, E_n , of anion, Na_nX ($n=1$ or 2), in the padding solution on the conversion of tyrosine and histidine for reaction of *Philosamia cynthia ricini* silk fibroin with 1.43 M PGE in cyclohexane at 75°C for 3 hr: tyrosine, $\odot$; histidine, $\circ$.

Fig. 8 Substituent effect of epoxides on the conversion of tyrosine for reaction of *Philosamia cynthia ricini* silk fibroin with various epoxides in ethanol at 60°C for 6 hr, using 1 N $Na_2S_2O_3$ aqueous solution as padding solution: ---line for 99% confidence; -.-.- line for 95% confidence

Fig. 9 Substituent effect of epoxides on the conversion of histidine for reaction of *Philosamia cynthia ricini* silk fibroin with various epoxides in ethanol at 60°C for 6 hr, using 1 N $Na_2S_2O_3$ aqueous solution as padding solution: --- line for 99% confidence; -.-.- line for 95% confidence

The substituent effect of various epoxides as shown in Tables 2-4 can be put into a quantitative form for the reaction with these amino acid residues in *Philosamia cynthia ricini* fibroin by use of the modified Taft linear free-energy relationship²²:

$$\frac{(\log W - \log W_0)}{\sigma^*} = \rho_p + \frac{\rho_s E_s}{\sigma^*} \quad (2)$$

where W_0 and W are the conversion of tyrosine or histidine in the reaction of a given compound for the same reaction of one of its substituted derivatives, and the parameters, σ^* and E_s , are the polar and the steric substituent constants, and ρ_s and ρ_p are the reaction constants as the measures for the susceptibility of the reaction to the polar and the steric effects of the substituent, respectively.

In Figures 8 and 9, $(\log W - \log W_0) / \sigma^*$ is plotted against E_s / σ^* for the reaction of a series of epoxides with tyrosine and histidine, respectively. The parent compound with the conversion of W_0 is PO, for which σ^* and E_s are both zero, and these values for the other epoxides are as follows²²:

BO, -0.100 , -0.070 ; G, $+0.555$, -0.170 ; E, $+1.050$, -0.24 ; PGE, $+0.850$, -0.33 ; SO, $+0.600$, -0.80 ; EGE, $+0.50$, -0.26 ; AGE, $+0.65$ (or $+0.60$), -0.30 .

Table 5. Values of ρ_s and ρ_p for reactions of tyrosine and histidine in *Philosamia cynthia ricini* silk fibroin with 1,2-epoxy-3-phenoxypropane (PGE)^a

	Temperature	ρ_s	ρ_p	r	n	ρ_s/ρ_p
<i>Philosamia cynthia ricini</i>						
Tyrosine	45°C	3.26	1.29	0.916	7	2.52
	53	2.97	1.13	0.910	7	2.63
	60	2.40	0.968	0.901	10	2.48
Histidine	45	2.66	1.56	0.873	7	1.71
	53	2.64	1.51	0.852	7	1.74
	60	1.97	0.946	0.835	10	2.09
<i>Bombyx mori</i> ^b						
Tyrosine	45	2.26	0.460	0.944	7	4.91
	50	2.57	0.772	0.962	7	3.33
	60	2.11	0.417	0.928	10	5.06
	68	2.01	0.373	0.970	8	5.39

a See equation 2 in the Text. Calculated from Data in Tables 3 and 4.

b Recalculated values using data in ref. 6

Es value for SO is tentatively estimated from that (-0.79) for cyclohexy; Es and σ^* values for EGE and AGE are those for CH_3OCH_2 and $\text{C}_6\text{H}_5\text{OCH}_2$, respectively. The best straight lines have been determined by the least-squares method and, from the intercept and the slope, ρ_p and ρ_s values are obtained as shown in Table 5. The positive value of ρ_p indicates that electron-withdrawing substituents increase the rate, which is usually interpreted to indicate that the reaction is $\text{S}_\text{N}2$, with bond-making more important than bond-breaking. The larger ρ_p values observed indicate that the steric factor in the substituents plays a more important role than polar factor in the epoxide reaction with tyrosine and histidine residues in *Philosamia cynthia ricini* fibroin, compared to the other epoxide reactions in homogeneous solutions. This suggests that adducts of the epoxide with these amino acid residues are not with the primary hydroxyl of $\text{RCH}(\text{CH}_2\text{OH})\text{CH}_2\text{R}'$, but the secondary one as $\text{RCH}_2\text{CH}(\text{OH})\text{CH}_2\text{R}'$, where R is an organic group such as $-\text{COO}$, $-\text{NH}$, $-\text{N}$, $-\text{O}$ of amino acid residue in fibroin and R' is a substituent of the epoxide, $\text{OCH}_2\text{CHCH}_2\text{R}'$. The latter type adducts of lysine has been found in the reaction of PO with *Bombyx mori* fibroin¹⁵. Moreover, the value of $\rho_s/\rho_p = 2.5$ – 2.6 for the tyrosine reaction is larger than that, 1.7 – 2.1 , for the histidine, showing that

the activated species of histidine should be of type Ia or Ib. The values for the tyrosine in *Philosamia cynthia ricini* is much smaller than those, 3.3 – 5.4 , in the *Bombyx mori* fibroin, and is associated with larger intermolecular distance and significant ability of the *Philosamia cynthia ricini* fibroin to swell with aqueous solutions⁷, although the reactivity of functional amino acid residues in fibroins may depend on the variation in the form of the samples, i.e., fiber or fabrics.

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エリ蚕絹フィブロインの官能性アミノ酸 側鎖に対するモノエポキシドの反応性

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触媒として中性塩水溶液を含浸したエリ蚕絹フィブロインと種々モノエポキシドとの不均一系付加反応をエタノールあるいはシクロヘキサン溶媒中、温度45~75℃で検討した。主要な官能性アミノ酸側鎖の反応性は次の順に低下した: His > Tyr > Arg. Tyr および Arg 側鎖の反応では含浸中性塩の濃度効果が顕著であったが、His や

Lys 側鎖の反応には中性塩濃度はほとんど影響しなかった。Tyr 側鎖の反応は含浸中性塩の陰イオンの求核性によって説明できた。更に Tyr および His 側鎖の反応に対するエポキシドの置換基効果を修正 Taft 式を用いて議論した。又家蚕絹フィブロインの反応性との差異を考察した。