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Studies on the Sorption and Permeation of Acid dyes through Silk fibroin Membrane (I)

—The structure of Silk fiber and fibroin Membrane—

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견 피브로인 막을 통한 산성염료의 흡착과 투과에 관한 연구(Ⅰ)

——견섬유와 피브로인 막의 구조——

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적 요

견 피브로인과 그 응용에 관한 기초적인 자료를 얻기 위하여, 본 연구에서는, 견섬유와 피브로인 막의 구조에 대해서 IR, DSC, X-ray diffraction, SEM, 아미노산분석 등의 조사를 통하여 구조변화를 비교하였다. IR과 DSC를 통하여 얻은 결과, 피브로인 막의 구조는 대부분 random coil과 Silk I type으로 구성되어 있지만, Silk II type의 존재도 인정되었다. 피브로인 막을 전자현미경으로 관찰한 결과, 표면은 구상체로 치밀하게 형성되어 있었고, 단면구조는 거의 대칭적인 것임을 알았다. 견섬유와 피브로인 막의 아미노산 분석결과, 전체에 대한 염기성 아미노산의 함량은 피브로인 막보다 견섬유가 더 많은 것을 알 수 있었다.

I. Introduction

Generally, the protein molecules have three conformations namely, random coil, α-helix, and β-sheet. In the case of silk fibroin, it has been known that there are two types of crystalline structure (Hirabayashi et al., 1967; Konishi et al., 1968), Silk I type, crank shaft conformation and Silk II type, anti-parallel β-pleated sheet conformation. Many investigations for the conformation of silk in the natural fiber and in regenerated films have been made(Tashiro et al., 1970; Sasaki et al., 1974; Ishikawa et al., 1983). The molecular structure of silk fibroin can be complicatedly transformed by mechanical shear force, heat, organic solvents, and casting condition (Bhat et al., 1980; Magoshi et al., 1977).

In general, when silk fibroin aqueous solution is cast, Silk I type is obtained below 40°C, whereas Silk II type is obtained above 40°C (Hirabayashi et al., 1968a; Aoki et al., 1974). Magoshi et al.(1977) have known that the conformational transitions from random coil to Silk II type and from α-helix to Silk II type are induced by heat treatment at 220°C.

In order to elucidate the molecular conformation of silk fibroin, Tstukada(1985) investigated structural changes on casting conditions. Namely, the lower the casting speed, the more the silk fibroin molecules gave a beta-rich conformation even if the casting temperature is high, and the higher the casting speed, the more the silk fibroin molecules gave a random coil-rich form. This indicates that the molecular conformation depends not only on the casting temperature but also on the casting speed.

Nakajima et al. (1983) investigated into dialysis conditions for obtaining Silk I type from crystalline fraction of silk fibroin, and knew what initial pH of silk fibroin solution dissoved by lithium bromide aqueous solution and initial pH of dialyser were both above pH7, the molecular structure was transformed Silk II type conformation, when both below pH 5, that was transformed Silk I type conformation.

Bhat et al.(1983) have suggested that the transfor mation to Silk II type could be brought about by heating, solvent induced crystallization, UV radiation, and prolonged storage. It has been pointed out that a rapid transformation to Silk II type takes place under UV radiation. On the contrast, γ-irradiation leads to decrystallization. The mode of transformation to Silk II type differs in simple drying conditions and under UV radiation or sunlight. And it is known that the film regenerated from lithium thiocyanate aqueous solution behaves differently from that regenerated from the silkworm gland.

In a recent study, Nagura et al. (1984) described a fine structure of silk fibroin film from silkworm gland. Silk I type film is composed of large number of Silk I type crystals and random coils, and small number of α-helical and Silk II type chains, which are dispersed in the random coils. Silk II type film formed by heating above 220°C and then by annealing at this temperature is composed of large numbers of Silk II type crystals and random coils, and small numbers of α-helical chains dispersed in the random coils. Silk II type crystals are crystallized in the cooling process after a disordering of the Silk I type crystals by cutting of the intermolecular hydrogen bonds above 220°C.

Unfortunately, Studies on the morphology of surface and cross-section of regenerated silk fibroin membrane were hardly reported. Moreover, it was scarcely investigated that whether the compositions of amino acid of silk fiber and regenerated silk membrane are the same or not.

In this study, the structure of silk membrane was investigated by means of X-ray diffraction measurement, infrared spectrometer, differential scanning calorimeter and scanning electron microscope. The amino acid analysis was also studied.

II. Experimental

1. Materials

The commercial raw silk fibers(21D) were degummed by the following method. The raw silk fibers were treated with 15% (o.w.f.) marseilles soap and 10% (o.w.f.) sodium carbonate aqueous solution for 2 hours at 95°C (L.R=50 : 1). The fibers were washed with boiling water, rinsed with water for several times, and finally dried at room temperature. The degumming ratio was 24.8% and the degummed silk fibers were purified by soxhelet apparatus with ethanol and ether for 30 hours, respectively.

2. Membrane preparation

The degummed silk fibers were dissolved in the aqueous solution of lithium bromide saturated for 1 hour at about 37°C with shaking, and the 10% silk fibroin solution was prepared. The mixed solution was provided by dissolving 60g lithium bromide in 40g of distilled water. After removing the undissolved or precipitated part by filtering (glass filter 3G3) under reduced pressure, the silk fibroin solution was dialyzed against distilled water for 2 days at 20°C, using cylindrical cellulose dialysis sack (sigma, 250-9u). The completion of desalting was confirmed by means of conductivity meter (Jenway PCM 3). By weighing the silk fibroin solution, it was found that the silk fibroin solution was diluted to about 5% concentration during the dialysis. The dialyzed silk fibroin solution was cast onto commercial acryl plate and dried under room temperature for 2 days. The membrane just after dried was soluble in the water. For nonsoluble form, the membrane was treated with methyl alcohol at 25°C for 2 hours, washed with water thoroughly, and dried at room temperature.

3. Instrumental analysis

The IR spectra were recorded with a Bruker IFS-85, FT-IR in the range of 4000-400cm^{-1}.

Differential scanning calorimetry was performed on a Dupont 9900 DSC V2·2A under N_2 gas and the analytical conditions were as follows;

Heating rate, 5 degree/min.

Sample weight, about 3mg

Standard material, aluminium oxide

Silk fiber was cut into fine fractions and seived. A pellet was used for the X-ray diffractometric study and silk membrane was used directly. The X-ray generator (model D/MAX-IIA, Rigaku with diffractometric arrangement) and Ni-filtered Cu K_α radiation were used.

The samples for SEM micrograph were coated with gold and observated by means of scanning electron microscope (Aakashi-DS 130).

The sample was hydrolyzed with 6N hydrochloric acid for 24 hours at 105°C to prepare for amino acid analysis. The amino acid analysis was done with amino acid analyzer (Biotronik LC 5001).

III. Results and Discussion

The information of a fine structure of silk fibroin has been investigated earlier by IR spectra. (Hirabayashi et al., 1968b). Amide I, II, III, and V bands, the characteristic infrared ones of proteins, appeared at 1700~1600cm^{-1}, 1570~1500cm^{-1}, 1300~1200cm^{-1}, and 700~630cm^{-1}, respectively. The characteristic bands assigned to each conformation were different with different investigators as well as with different kinds of silk fibroin.

Kondo et al. (1967) have pointed out that the characteristic bands of α-helix appeared at 1650, 1545, 620cm^{-1} as amide I, II and V, random coil appeared at 1655, 1535, 650cm^{-1} and β-sheet appeared at 1630, 1530, 690cm^{-1} respectively. Hirabayashi et al. (1976) reported the bands at 1630, 1530, 1265, and 700cm^{-1} as amides I, II, III, and V assigned for β-sheet conformation of Mulbery Silk and those at 1660, 1540, 1235, and 650cm^{-1} as the ones assigned for random coil conformation. For crystalline part of silk fibroin, the bands at 1635 and 1530cm^{-1} were assigned for Silk II type conformation corresponding to amide I and II, respectively, and Silk I type silk fibroin converted by treatment of lithium bromide showed that amide I and II bands appeared at 1660~1665cm^{-1} and 1531cm^{-1}, respectively. It was indicated that the bands at 1266 (w), 1233(m)cm^{-1} as amide III assigned for Silk II type conformation and those at 1266(w), 1240(m)cm^{-1} as the ones assigned to Silk I type conformation (Hirabayashi et al., 1968c). In

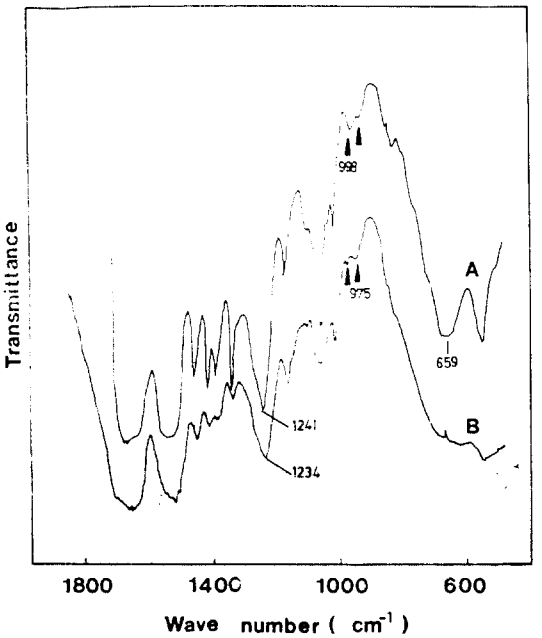

Fig. 1. Infrared spectra of silk fibroin fiber (B) and membrane (A).

the polypeptide (Gly-L-Ala), the bands at 998 and 975cm^{-1} assigned for Silk II type conformation.

In Fig. 1, it shows that the amide I and II bands of silk fibroin fiber are duplicated, however those of silk fibroin membrane imply the random coil or Silk I type conformation. The band at 1234 and 1241cm^{-1} as amide III of silk fibroin fiber and membrane exhibit the Silk II type conformation and Silk I type conformation, respectively. The characteristic bands of Silk II type conformation at 998 and 975cm^{-1} were appeared at both silk fibroin fiber and membrane. The band at 695cm^{-1} as amide V band of membrane assign the random coil conformation.

On the other hand, Bhat et al. (1983) pointed out that the amide III bands at 1265 and 1235cm^{-1} were suitable to calculate the crystallinity of silk fibroin quantitatively. IR crystallinity indices were calculated by the ratio of optical density at 1265 and 1235cm^{-1} IR crystallinity indices of silk fibroin fiber and membrane were 0.7616 and 0.6073, respectively. It was found that silk fibroin fiber consisted of mostly Silk II type conformation and silk fibroin membrane was composed of the Silk I type and the Silk II type

conformation. However, it was supposed that the structure of silk fibroin membrane had mostly random coil and Silk I type conformation.

In order to investigate the structure of silk, the thermal behaviour of silk has been studied by several workers (Ishikawa et al., 1972; Hirabayashi et al., 1974). According to the DSC thermograns, it was reported that three peaks were appeared on the DSC curves of silk, that is, first peak arised at about 100°C, second at about 240°C and third above 300°C. The first peak is attributed to water evaporation, the second corresponds to $\alpha-\beta$ transition of coagulated fibroin and the third displays the decomposition of non-oriented Silk II type fibroin and high oriented Silk II type fibroin. However, the peaks can be shifted more or less depending on variuos measurement conditions such as sample weight and heating rate (Ishikawa et al., 1972). As the $\alpha-\beta$ transition of coagulated fibroin takes place at about 240—250°C, the resulting Silk II type fibroin is decomposed at about 290~300°C.

Fig. 2 shows the DSC curves of silk fibroin fiber and membrane. On the DSC curve of silk fiber, the peak attributed to the decomposition of Silk II type fibroin takes place at 306°C. On the other hand, silk fibroin membrane shows the endothermic peaks at 284 and 293°C on the DSC thermogram, in addition several shoulder which can be considered to be caused by $\alpha-\beta$ transition observed at around 250°C.

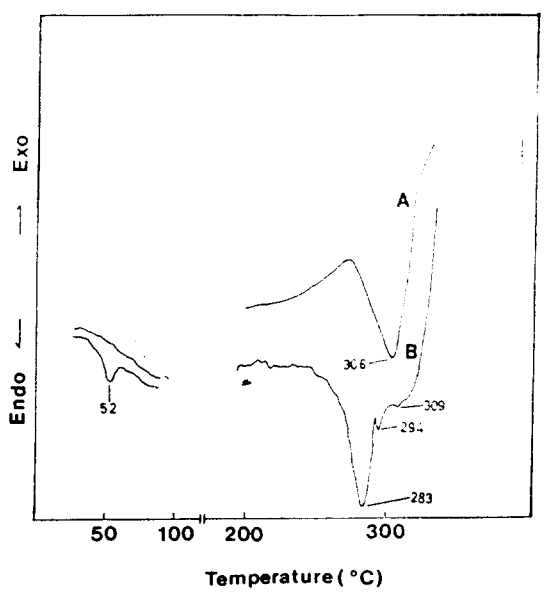

Fig. 2. DSC thermograms of silk fibroin fiber (A) and membrane (B).

In general, it is known that liquid silk fibroin molecule converted from randomly coiled Silk I type to structed Silk II type at about 50°C, and the silk fibroin molecule in solid state converted its structure at about 200°C (Magoshi et al., 1977; Kataoka, 1974).

In silk fibroin membrane, the endothermic peak appeared at 52°C, while in silk fibroin fiber no such a peak appeared. It might be considered that this peak attributed to the partial change from the Silk I type, which is similar state to the liquid silk fibroin because of the water, to the Silk II type. It was reported that endothermic peak occurred at 54°C in native Tussah silk fibroin, and this peak was attributed to the $\alpha-\beta$ transition which was proved by means of X-ray diffraction. These results obtained from DSC thermogram indicate that the silk fibroin membrane was mostly composed of the random coil and Silk I type conformation.

The analysis of IR spectra and DSC thermograms revealed that silk fibroin membrane mostly consisted of random coil and Silk I type configuration. However, it is worthwhile to check these conclusions by using X-ray diffraction method. The X-ray diffraction curves of silk are shown in Fig. 3. It can be seen from the curve that no sharp peak corresponding to crystalline portion is apparent in the silk fibroin membrane. This indicates that the silk fibroin membrane are mostly made up of the random coil configuration. However, it was revealed in IR spectra that the charicteristic band of the Silk II type at 998 and 975cm^{-1} were appeared in the silk membrane, too. The peak corresponding to crystalline portion in IR spectra may not be apparent in X-ray diffraction curve, since IR spectra spectroscopy is sensitive to short-range order while the X-ray diffraction is sensitive to long-range order. In the results obtained from IR spectra, it is considered that silk fibroin membrane has short-range order but probably because of the small size of the crystallities, it is hardy reflected in the X-ray diffraction curve.

In the X-ray diffraction pattern of silk fibroin fiber, the peaks at 2θ of 20.3° and 16.1° develope well. These peaks, according to Bhat et al. (1983), correspond to the d(201) and d(002) of Silk II type crystal, respectively. Therefore, the results obtained from X-ray diffraction patterns are coincident with those from IR spectra.

The degree of crystallinity (Xc) is equal to the ratio of the integrated crystalline scattering to the total scattering (both crystalline and amorphous) and is given by

$$X_c = \int_0^\infty S^2 Ic(s)\,ds / \int_0^\infty S^2 I(s)\,ds \qquad (1)$$

where S describesthe magnitude of the reciprocal-lattice vector which is given by

$$S = (2\sin\theta)/\lambda \qquad (2)$$

θ means one-half the angle of deviation of the diffracted rays from the incident X-rays. λ is X-ray wavelength, $I(s)$ is the intensity of coherent X-ray scatter from a specimen (both crystalline and amorphous). The degree of crystallinity of the silk fibroin fiber and the membrane obtained were 0.54 and 0.44, respectively. These values were lower than the IR crystallinity indices. The degree of crystallinity from eq. (1) tends to be smaller than the true crystalline fraction, because a part of the X-ray intensity which is scattered by the crystalline regions is lost from the peaks and appears as diffuse scatter in the background as a result of atomic thermal vibrations and lattice imperfections.

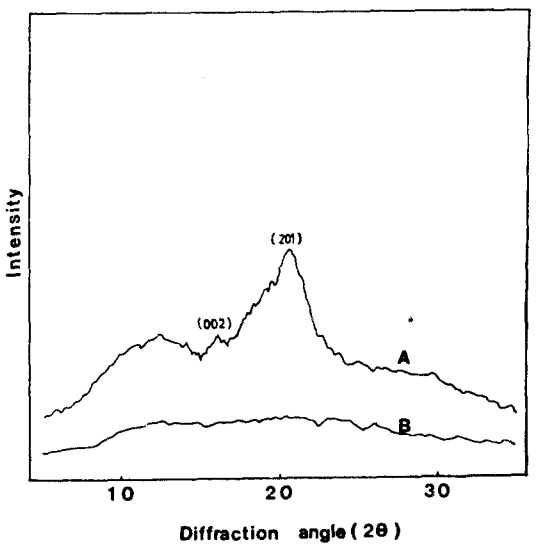

Fig. 3. X-ray diffraction curves of silk fibroin fiber (A) and membrne (B).

The SEM micrograph of the silk fibroin membrane is shown in Fig. 3. The surface structure of silk fibroin casted was discussed by Tsukada and in his observation, it was pointed out that the spherulites from the silk fibroin solution(0.4%) were shown at the surface of the membrane. In this study, the spherulites were compacted at the surface of the membrane(A). The spherulite size was about 0.3μm. This form is supposed to be a preliminary stage of fibrillar one appeared in silk fiber. In the case of bottom side of the membrane(B) the spherular form was not observed. It is thought that the growth of spherular from was hindered by the castingplate. The cross-sectional form(C) was shown almost symmetrical structure.

It has been known that silk fibroin is mostly composed of glycine and alanine. The composition ratio of amino acid differs by investigators (Ishikawa et al., 1983). Table 1 shows the amino acid composition of the silk fibroin fiber and the membrane. The content of basic amino acid residues for the total amino acid in the fiber is larger than that in the

Table 1. The amino acid compositions of the silk fibroin fiber and the membrane (g/100g)

Amino acid	Fiber	Membrane
Glycine	33.18	32.87
Alanine	27.33	27.03
Serine	10.96	11.10
Tyrocine	8.83	8.78
Aspartic acid	3.28	3.01
Valine	3.20	3.00
Glutamic acid	2.39	2.30
Isoleucine	2.03	1.98
Histidine	1.54	1.05
Phenylalanine	1.52	1.37
Threonine	1.35	1.23
Leucine	1.04	0.87
Lysine	0.97	0.61
Proline	0.71	0.74
Arginine	0.67	0.77
Metionine	0.47	0.49
Total	99.47	97.20

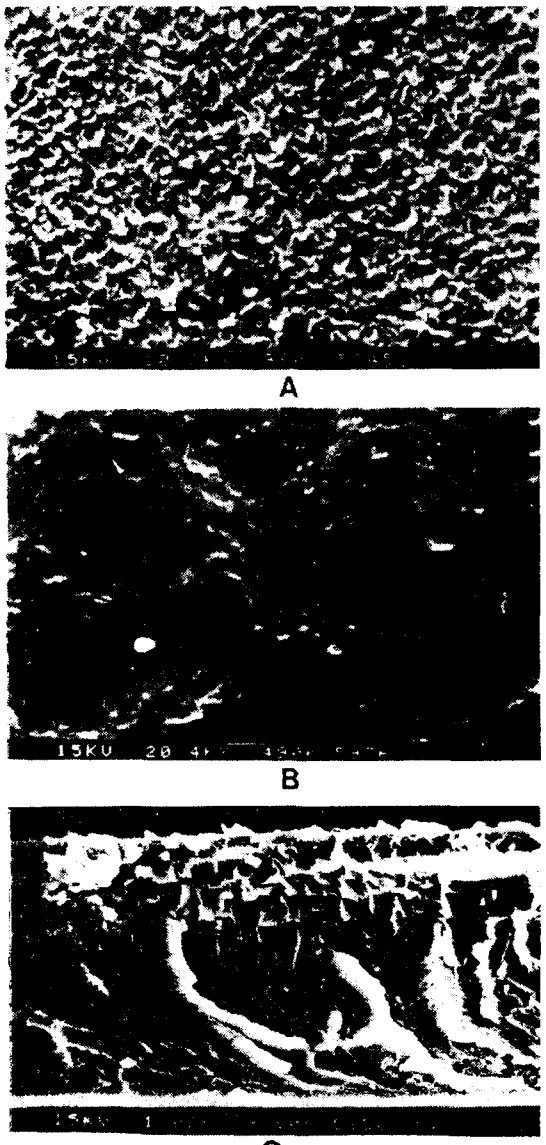

Fig. 4. The SEM micrograph of silk fibroin membrane. (A); surface (B); Bottom (C); cross-section

membrane. And, in general, the mole ratio of glycine and alanine residues for the total amino acid is about 70% as pointed out by other investigators, but in this experiment it is about 60% in both the fiber and the membrane. It can be considered that the dissolved amino acid was mixed with high molecular weight molecules and low molecular weight melecules in the process of dissolving the silk fiber by lithium bromide solution. Thus, low molecular weight molecules can be removed in dialysis process, the composition of amino acid may change. More detail study on the difference of amino acid composition is required in future. In the fact that the change of amino acid composition influences the dye uptake, that is very important subject.

IV conclusion

This study was undertaken to establish some basic essentional informations for silk fibroin. The structure of silk membrane in relation to that of silk fiber was investigated by the instrumental analysis.

1. The amide III bands of silk fiber and membrane in IR spetra appeared at 1234 and 1241cm^{-1}, and IR crystallinity indices were found 0.7016 and 0.6073, respectively.

2. In the DSC thermogram of silk fiber, the peak at 306°C attributed to the decomposition of Silk II type fibroin. The endothermic peaks were observed at 52°C, 284°C, 293°C, and 309°C on the DSC thermogram of silk membrane. The peak at 52°C attributed to the partial change from Silk I to Silk II type.

3. The X-ray diffraction showed that peaks of silk fiber at 2θ of 20.3° and 16.1° were well developed, while no sharp peak was found in silk membrane. The degree of crystallinity of silk fiber and membrane were 0.54 and 0.44, respectively.

4. SEMs of silk membrane showed that the spherulites were compact at the surface of membrane and was about 0.3μm in size. The cross-sectional form was found almost symmetrical in shape.

5. The contents of basic amino acid residues for the total amino acid in fiber were larger than those in membrane.

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