Chemistry and Structure of Silk

By KEI-ICHI KOMATSU

Division of Chemistry, Sericulture Experiment Station

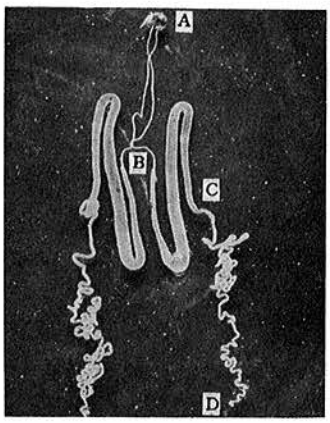
Silk, which has been utilized widely as an excellent textile fiber, has also been used as a good material for research, as a typical protein. Numerous studies on silk, carried out continuously over a period of more than one century, have contributed not only to the improvement of production process and quality of raw silk and silk products, that has supported the development of sericulture and silk industry, but also to the progress of research on protein and fiber.

In the present paper some of the recent studies selected from the research works in Japan on the chemistry and structure of silk will be described briefly.

Mechanism of cocoon fiber formation

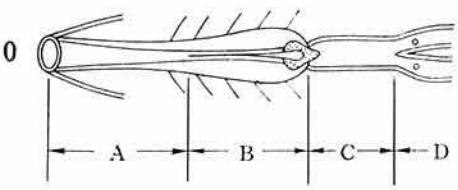
It has been known since old time that the cocoon fiber is formed by the insolubilization caused only by a mechanical action, the spinning, from the liquid silk stored in silk glands of matured silkworms. The insolubilization by the mechanical action is a characteristic of fibroin, that can be referred to mechanical denaturation, and is not known yet with natural and artificial high polymers including proteins.

It was already pointed out from the viewpoint of molecular theory that the mechanism of fiber formation of liquid silk fibroin can be approached as a problem of unfolding molecular chains due to the shear stress caused by the passing of liquid silk inside silk glands. In this connection, further studies have been carried out on the molecular conformations of fibroin and sericin, and their changes at each part of the silk gland (Plate 1) were studied in detail, and it was made clear that the fiber formation by the spinning of liquid silk fibroin proceeds by two steps[5]. In the first step, the critical shear rate required for β-transition (unfolding of molecular chains) is lowered from 2×10^{2}/sec to 1×10^{-1}/sec, due to a decrease in water content of amorphous liquid fibroin from about 84% to about 75% that occurs as the fibroin proceeds from the posterior division to the middle division, and then, as soon as the fibroin flows into the anterior division, nuclei for the β-form are produced, creating a pre-state of three dimensional network, so



A～B : Anterior division
35～40mm, 0.05～0.3mmϕ
B～C : Middle division
60～65mm, 1.2～2.5mmϕ
C～D : Posterior division
200～250mm, 0.4～0.8mmϕ

Plate 1. Silk gland of matured silkworm larva (*Bombyx mori*)



A: Spinneret
B: Silk Press
C: Joined duct
D: Posterior division
O: Orifice of spinneret

Fig. 1. Schematic representation of spinneret of silkworm

that the fibroin is oriented along its flowing direction. The second step takes place at the silk press of spinneret (Fig. 1), the most narrow part in the whole course of liquid fibroin flow, where a large shear stress applied to the liquid fibroin causes unfolding of folded molecular chains of liquid fibroin, with the occurrence of β-transition and crystallization, thus completing the fiber formation.

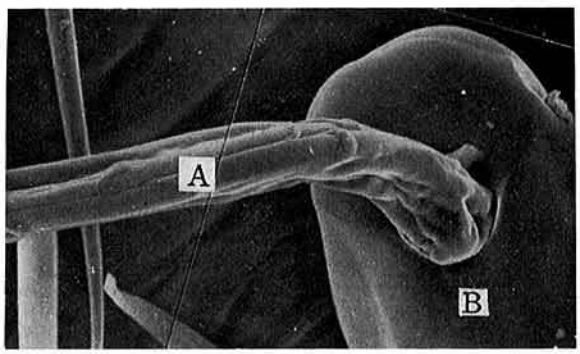
In that process, sericin, another important component of cocoon fibre, is not crystallized, in spite of the fact that sericin flows from the middle division to the spinneret through the anterior division in the manner as if it encloses fibroin. The reason is that the liquid silk sericin contains about 86% of water and requires a critical shear rate as large as 10^4 times that of fibroin for unfolding its molecular chains. Accordingly, sericin serves as a lubricant to facilitate the formation of cocoon fiber, being present between silk gland cells and fibroin.

The appearance of cocoon fiber by spinning was directly observed by the cryo-scanning electron microscopic method[13] (Plate 2).

Dissolution behavior and structure of sericin

Although there are many arguments whether sericin is composed of a single protein or not, it seems that it is composed of more than one component, judged at least from its dissolution behavior to hot water. As the dissolution to hot water is an important practical problem, being closely related to the reelability of cocoon and the production process of raw silk, studies have been made in relation to high order structure[8].

The dissolution curve determined by the UV absorption method showed 3 bends, indicating that cocoon fiber sericin is composed of 4 different fractions different in dissolution



A: Spun cocoon fiber

B: Labium

Plate 2. Spinning of cocoon fiber by matured silkworm (*Bombyx mori*)

velocity. These 4 fractions, designated sericin I, II, III and IV in the order of easiness of dissolution, are distributed almost as a stratified structure from the surface to the inner portion of liquid silk and cocoon fiber. The innermost layer, sericin IV, constitutes a thin wall together with cocoon wax, contained at 20–30%, to separate sericin distinctly from fibroin, and is related to the formation of cocoon fiber by spinning and to the feeling

Table 1. Amino acid composition of sericin fraction prepared by fractional dissolution with hot water from cocoon filament (mol %)

Amino acid	Sericin fraction				Whole sericin
	Sericin I	Sericin II	Sericin III	Sericin IV	
Gly	13.21	12.81	15.69	11.89	13.49
Ala	4.68	6.69	6.68	9.30	5.97
Val	2.97	2.21	3.21	4.16	2.75
Leu	0.86	0.96	1.27	6.26	1.14
Ile	0.59	0.57	0.85	3.50	0.72
Pro	0.58	0.63	0.66	2.75	0.68
Phe	0.45	0.44	0.50	2.83	0.53
Try	0.19	0.20	0.25	0.23	0.21
Cys	0.17	0.15	0.12	0	0.15
Met	0.04	0.04	0.04	0.12	0.04
Ser	34.03	36.64	28.15	12.40	33.43
Thr	10.34	8.48	11.36	7.25	9.74
Tyr	2.53	2.43	3.15	2.45	2.61
Asp	16.94	16.95	16.13	12.64	16.71
Glu	4.73	3.64	4.09	11.32	4.42
Arg	3.20	2.65	3.68	3.93	3.10
His	1.25	1.22	1.49	1.87	1.30
Lys	3.28	3.29	2.64	7.11	3.30
Total of oxyamino acids	46.90	47.54	42.66	22.10	45.78
Total of acidic amino acids	21.67	20.59	20.22	23.96	21.13
Total of basic amino acids	7.73	7.16	7.81	12.91	7.70
Total of amino acids with polar side chain (Ap)	76.30	75.29	70.69	58.97	74.61
Total of amino acids with nonpolar side chain (An)	23.74	24.70	29.27	41.03	25.68
Ratio (Ap/An)	3.21	3.05	2.42	1.44	2.91

Table 2. Properties of sericin fractionated with hot water

		Sericin I	Sericin II	Sericin III	Sericin IV	Whole Sericin
Content	(%)	41.0	38.6	17.6	3.1	100
Coefficient of dissolution velocity		5.33	1.76	0.70	0.22	—
Moisture regain	(%)	16.7	16.2	15.7	14.5	16.3
Specific gravity		1.400	1.403	1.408	1.412	1.407
Crystallinity	(%)	3.0	18.2	32.5	37.6	15.06

and handling of silk products.

There are differences in amino acid composition among the 4 fractions of sericin[8] (Table 1), but they are not large enough to explain the difference of dissolution to hot water. The high order structure is considered to be more influential to the dissolution to hot water. In fact, experiments using infrared absorption and X-ray diffraction, etc. have proved that major molecular conformation of easily dissoluble sericin is random coil, whereas the more difficult the dissolution, the more is the β-conformation, the higher is the specific gravity and degree of crystallization, and the denser is the molecular aggregating structure (Table 2). With the easily dissoluble sericin, rich in random coil, it was found out that the β-transition and crystallization are promoted, and molecular aggregating structure becomes dense, resulting in a decreased dissolubility, by the repeat of wetting and drying the sericin. This phenomenon gave many useful suggestions as to the handling of fresh cocoons, drying and storage of cocoons. On the other hand, it was also found that apparent molecular weight is decreased when sericin is heated at the presence of large amount of water. By utilizing this principle, methods of improving the quality of cocoons with poor reelability were deviced.

By mechanical stretching, the high order structure of sericin can be changed to the fibre structure with antiparallel chain pleated sheets of β-conformation oriented along the stretching axis and with the fibre period of 6.84 ± 0.02 A. However, this structure is unstable, returning easily to the unoriented structure by hot water treatment. Based on the change of high order structure by mechanical stretching and the elastic behavior of stretched sericin in wet, a model like a globule with crystal region enclosed by amorphous region of the molecular chains was proposed for the molecular aggregating structure of sericin[8]. The fact that sericins with varying dissolubility to hot water are different in their higher order structure and that the easily dissoluble sericin becomes to be difficult to dissolve due to an increased density of molecular aggregating structure by wetting and drying was supported by thermal analysis[4]. It was also pointed out that the slow drying of wetted sericin which promotes crystallization and lowers dissolubility is a reason for poor reelability of cocoons produced under high temperature and humid environments[4,6].

Sericin is absorbed by powdered active carbon following the Freundlich's absorption isotherm equation. Easily dissoluble sericin is more easily absorbed than less dissoluble ones, suggesting the difference in high order structure of them. Based on the properties of poly-α-amino acids, synthesized with 8 major amino acids of sericin at the molar ratio similar to sericin, it was presumed that characteristics of sericin are more closely related to amino acid sequence than amino acid composition. Absorption of the poly-α-amino acids by powdered active carbon also follows the Freundlich's equation and higher molecular weight gives easy absorption[17].

Wild cocoons are generally low in dissolubility to hot water, but no remarkable differences were observed by species of silkworm (Table 3), and it was concluded that the low dissolubility is caused by secondary components contained in sericin and the dense physical structure of cocoon shells[9].

Amino acid composition and chemical structure of fibroin

Characteristics in amino acid composition of fibroin are that amino acids with short side chains like glycine, alanine and serine are abundant, reaching more than 80% when tyrosine is included, and that amino acids with polar side chains are of small amount. Interesting is that the amino acid composition of fibroin is apparently specific to silkworm species, so that fibroin can be classified accordingly. Similarly, the chemical structure (amino acid sequence) of peptide chains constituting crystal region can be classified cor-

Table 3. Amino acid composition of domestic and wild cocoon sericin (mol %)

Amino Acids	*Bombyx mori*	*Antheraea*				*Philosamia cynthia ricini*
		yamamai	*pernyi*	*mylitta*	*assama*	
Gly	12.70	15.32	14.99	14.91	15.28	11.88
Ala	5.51	2.27	2.78	2.73	2.75	4.38
Val	2.68	0.62	1.19	0.79	0.77	1.00
Leu	0.72	0.78	0.99	0.55	0.59	0.69
Ile	0.55	0.99	0.80	0.39	0.41	0.61
Pro	0.57	1.55	1.91	1.09	1.50	2.23
Phe	0.43	0.30	0.60	2.47	0.32	0.76
Try	—	—	—	—	—	—
Cys	0.14	0.21	0.18	trace	0.15	0.45
Met	0.05	0.07	0.13	trace	0.06	0.07
Ser	31.97	22.63	22.63	23.21	23.03	28.96
Thr	8.25	14.89	14.96	13.16	14.56	7.23
Tyr	3.40	5.14	4.92	4.33	4.64	3.70
Asp	13.84	13.86	12.25	14.15	14.20	13.29
Glu	5.80	6.07	6.74	6.03	6.25	8.11
Arg	2.86	4.93	5.45	6.11	5.55	3.23
His	1.30	2.41	2.50	2.41	2.37	2.97
Lys	3.26	1.87	1.47	2.01	1.71	4.20
Total of amino acids with nonpolar side chain (A*n*)	23.36	22.10	23.58	22.92	21.83	22.08
Hydroxy amino acids	43.62	42.89	42.51	40.70	42.23	39.89
Acidic amino acids	19.64	19.93	18.99	20.18	20.45	21.40
Basic amino acids	7.42	9.21	9.42	10.53	9.63	10.40
Total of amino acids with polar side chaie (A*p*)	70.68	72.03	70.92	71.41	72.31	71.69
Ratio (A*p*/A*n*)	3.03	3.26	3.01	3.11	3.31	3.24

responding to silkworm species[7] (Table 4). In Table 4, X represents amino acid residues other than glycine, i.e. alanine or serine residues in *Bombycidae*, and amino acid residues other than alanine, i.e. glycine residue in *Thaumetopoeidae*. Accordingly, the chemical structure of crystal region of fibroin can be expressed approximately as polyglycyl-L-alanine (or L-serine) for *Bombycidae*, poly-L-alanine for *Saturniidae*, and poly-L-alanyl-glycine for *Thaumetopoeidae*.

Molecular weight of fibroin in silk gland was determined by the sedimentation analysis and gel electrophoresis with results that the molecular weight should be regarded to be 3.5×10^5, consisted of two subunits of 1.7×10^5 [16], to be consisted of 2.5×10^4 subunit and 3.6×10^5 subunit[1] or $(3.65\pm0.2)\times10^5$ consisted of 3 subunits of 2.6×10^4 and 1 subunit of 2.8×10^5, combined at disulfide bonds[14]. Also, as N terminal amino acids 1 mol each of aspartic acid and serine was obtained per 1 mol of fibroin[15]. These results suggest that polypeptide chains constituting fibroin are not necessarily uniform, but further accumulation of many data will be needed before a definite conclusion is obtained.

Table 4. Amino acid composition and main chemical structure of various fibroins (g/100 g fibroin)

	Bombyx		*Saturniidae*					*Thaumetopoeidae*		
			Antheraea		*Philosamia cynthia*		*Dictyoproca*	*Anaphe*		
	mori	*mandalina*	*pernyi*	*yamamai*	*ricini*	*pryeri*	*japonica*	*moroneyi*	*infracta*	*reticulata**
Ala	32.4	32.0	50.5	49.5	50.5	46.8	43.0	62.8	61.5	64.2
Gly	42.8	42.6	23.6	22.7	27.8	27.9	18.9	41.3	28.6	27.7
Tyr	11.8	10.9	8.8	8.1	10.7	9.3	9.2	1.70	2.85	2.83
Ser	14.7	14.7	11.3	11.0	7.0	5.9	11.5	1.21	5.80	3.08
Asp	1.73	1.83	6.58	6.86	4.48	4.33	5.56	0.79	2.13	2.28
Arg	0.90	0.94	6.06	7.00	3.81	3.06	6.94	0.09	1.76	1.57
His	0.32	0.27	1.41	1.51	1.74	1.39	1.98	0.20	1.16	4.38
Glu	1.74	1.53	1.34	1.13	1.23	0.98	1.98	0.39	1.93	0.73
Lys	0.45	0.39	0.26	0.20	0.46	0.34	0.34	0.22	0.23	0.21
Val	3.03	3.29	0.95	0.94	0.58	0.70	3.38	0.32	0.54	0.46
Leu	0.68	0.62	0.51	0.52	0.50	0.46	6.44	0.38	0.91	0.90
Ile	0.87	0.84	0.69	0.60	0.68	0.60	0.88	0.85	0.40	0.20
Phe	1.15	1.20	0.52	0.36	0.35	0.26	0.63	0.30	0.28	0.25
Pro	0.63	0.68	0.44	0.48	0.55	0.43	0.53	0.21	0.86	0.57
Thr	1.51	1.20	0.69	0.85	0.72	0.97	1.03	0.63	0.52	0.50
Met	0.10	0.01	0.03	0.03	0.02	0.02	0.01	0.22	0.11	1.31
Cys	0.03	0.04	0.04	0.05	0.01	0.01	0.02	0.01	0.01	—
Try	0.36	0.40	1.41	1.63	0.70	0.52	2.20	0.24	0.51	—
	Gly>Ala		Gly<Ala							
			Ser>Tyr		Ser<Tyr		Ser>Tyr	More than 50% in Ala		
			Rich in Val and Leu							

Bombyx	*Antheraea*	*Philosamia cynthia*	*Dictyoproca*	*Anaphe*
	Saturniidae			
-G-X-G-X-G-X-		-A-A-A-A-A-A-		-A-X-A-X-A-X-

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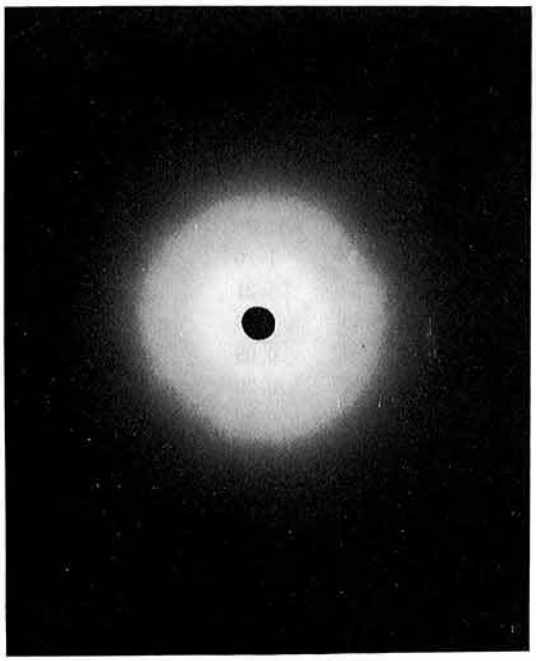
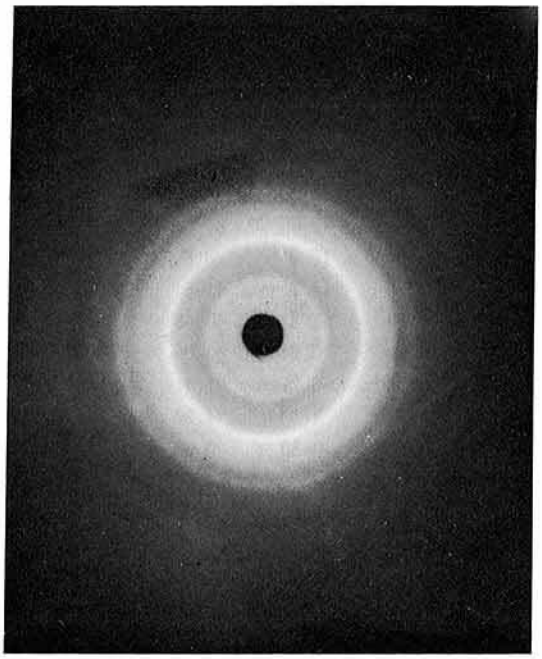
Crystal structure and transition of molecular conformation of fibroin

There are two kinds of fibroin crystals (Plate 3). The β-form (silk II) constituting silk fiber has been studied in detail and its whole picture was almost clarified. As to the α-form (silk I) obtained by the air drying below 50°C of liquid silk in silk glands, it is difficult to orient crystallites without causing the β-transition, so that analysis of its crystal structure has not progressed.

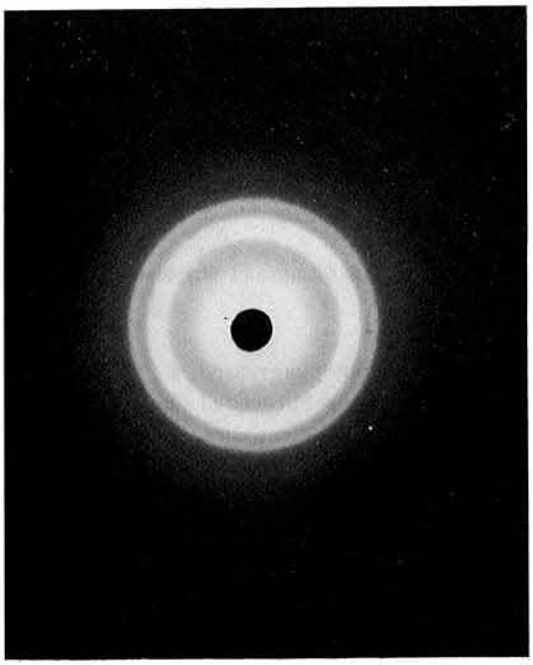
Recently it was succeeded in inducing the orientation without causing the β-transition by stretching the α-form fibroin membrane to which polyvinyl alcohol as a plasticizer was added. From a study in which the X-ray diffraction pattern and infrared absorption spectrum of this oriented membrane were analysed in comparison with those of polyglycyl-L-alanine used as a model of crystal region of fibroin[2], and a study in which the X-ray diffraction pattern, electron diffraction pattern and infrared absorption spectrum of α-form sediment mat acquired by recrystalli-



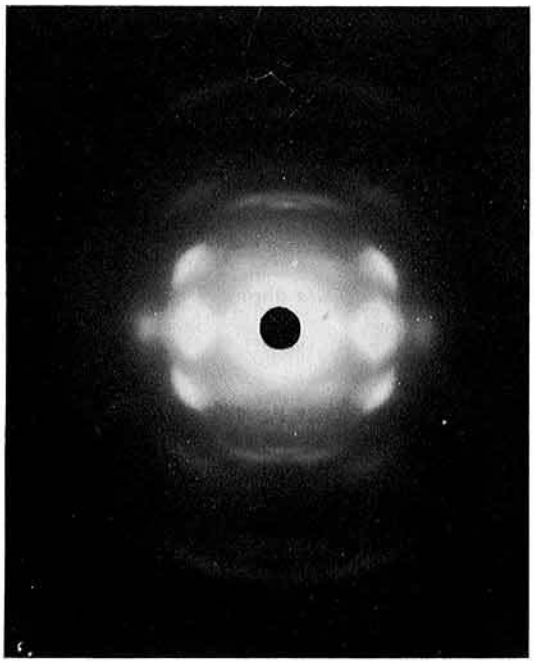
(A) Amorphous (by air-drying from fibroin solution)



(B) α - form (by air-drying from liquid fibroin)



(C) β - form (by hot - drying from liquid fibroin)



(D) Oriented β - form (silk fiber)

Plate 3. X-ray diffractions of fibroin (*Bombyx mori*)

Table 5. Dimensions of unit cell in α form fibroin (*Bombyx mori*)

	Crystal system	a (H-bonding)	b (Fiber axis)	c (Side chain)	n	t
Hirabayashi et al.[2]	Orthorhombic	4.45A	28.0A	7.16A	12	2.3A
Konishi et al.[10]	Orthorhombic	4.59	9.08	7.20	4	2.27

n: Number of amino acid residue in a unit cell
t: Residue translation

zation of crystal region, which was obtained from a regenerated fibroin solution by the enzymatic decomposition with α-chymotrypsin, were analysed[10], dimensions of unit cell of the α-form fibroin crystal were determined independently (Table 5). The results of these two studies differ each other as to the fibre period and number of amino acid residues in unit cells, recognized differently. Nevertheless, it was made clear from both results that the α-form of fibroin is a crystal consisted of molecular chains with a specific helical conformation connected by secondary force, and that the helical conformation is being more stretched than α-helix but less stretched than the β-form.

When fibroin solution is subjected to drying, the crystallization begins at a concentration of 65%, and at the drying temperature of 0–45°C the α-form is formed while at the temperature higher than 50°C the β-form[11]. Organic solvents, which cause the β-transition of random coils of fibroin, are required to have large dipole moment and dehydrating property but not to break molecular chains. Velocity of β-transition is markedly promoted when the organic solvents contain 20–40% of water and at temperature higher than 20–30°C. Furthermore, the randomly coiled fibroin membrane gives neither α- nor β-transition by the dry heating at the temperature of 40–180°C, but at the temperature higher than 190°C, it gives conformational transition to the β-form, while by the wet heating at 100–130°C, a part of it becomes the α-form and another part the β-form[12].

A study was made on the transition of fibroin molecule by the use of LiBr, a neutral salt frequently used to prepare regenerated fibroin solution from silk fiber without breaking molecular chains of fibroin[3]. Fibroin fiber shrank in the LiBr solution of 6 or higher M, extended on the contrary at 7.5 or higher M, and was broken at 8.5 or higher M at the temperature higher than 75°C. In the course of this process, molecular conformation of fibroin was transformed from the β-form to α-form, and further to random coil form, which showed a rubber-like elasticity. When LiBr was removed from the shrank fibroin by washing, it expresses the behavior similar to liquid fibroin in silk glands and when it was dried at room temperature it took the crystal structure of α-form. It is known from this result that LiBr plays a catalytic role in the α→β transition of fibroin.

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