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THERMAL PROPERTIES OF *BOMBYX MORI* AND SEVERAL WILD SILKWORM SILKS
Phase transition of liquid silk

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Abstract

The thermal properties of liquid silk from domestic and wild silkworms are investigated. Liquid silks obtained from the silk gland of the domesticated silkworm, *Bombyx mori* and four wild silkworms, *Samia cynthia ricini*, *Dictyoploca japonica*, *Antheraea pernyi* and *Antheraea yamamai* were used. The DSC curves for the liquid silk from the domestic silkworm have weak endothermic peaks corresponding to the breaking of hydrogen bonds in the α-form or to the untangling of physical network. The DSC curves for the wild silkworm silks, however, show clear exothermic peaks corresponding to a phase transition from the α-helix conformation to the β-form. Liquid silk from all the different silkworms undergoes a characteristic irreversible phase transition.

Keywords: domestic silkworm, liquid silk, phase transition, wild silkworm

Introduction

The silkworm as well as other insects, including bees, butterflies and spiders, produced silk fibers. Silkworms construct a cocoon from a self-produced long double monofilament to protect themselves during metamorphosis. Silk fibers are formed out of proteins synthesized from amino acids assimilated from the leaves of mulberry trees and stored in a pair of silk glands. This stored liquid silk is a highly viscous aqueous solution, or highly concentrated gel, comprising two distinct proteins, fibroin and sericin. These two proteins are synthesized in the posterior and middle divisions of the silk gland, respectively.

The mechanism of fiber formation from the liquid silk in the silk gland of the silkworm was first reported by Hiratsuka [1] and Foá [2]. They found that shear

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stresses and elongation, caused by the way the silkworm draws its head, are an integral part of the silk formation process. The fibers are formed from a nematic liquid crystalline state, which is then transformed via a gel state to the thread spun by the silkworm, essentially the product of a liquid-crystal spinning process [3].

Table 1 Amino acid composition and main chemical structure of various fibroin (g/100 g fibroin)

	Bombyx mori	*Samia cynthia ricini*	*Dictyploca japonica*	*Antheraea pernyi*	*Antheraea yamamai*
Ala	32.4	50.5	43.0	50.5	49.5
Gly	42.8	27.8	18.9	23.6	22.7
Tyr	11.8	10.7	9.2	8.8	8.1
Ser	14.7	7.0	11.5	11.3	11.0
Asp	1.73	4.48	5.56	6.58	6.86
Arg	0.90	3.81	6.94	6.06	7.00
His	0.32	1.74	1.98	1.41	1.51
Glu	1.74	1.23	1.98	1.34	1.13
Lys	0.45	0.46	0.34	0.26	0.20
Val	3.03	0.58	3.38	0.95	0.94
Leu	0.68	0.50	6.44	0.51	0.52
Ile	0.87	0.68	0.88	0.69	0.60
Phe	1.15	0.35	0.63	0.52	0.36
Pro	0.63	0.55	0.53	0.44	0.48
Thr	1.51	0.72	1.03	0.69	0.85
Met	0.10	0.02	0.01	0.03	0.03
Cys	0.03	0.01	0.02	0.04	0.05
Try	0.36	0.70	2.20	1.41	1.63
	Gly>Ala		Gly>Tyr		
		Ser<Tyr		Ser>Tyr	
				Asp, Arg	
			Val, Leu		
	-G-X-G-X		-A-A-A-A-		

A definitive difference between the domestic silkworm, *B. mori*, and wild silkworm fibroins is the amino acid composition of crystalline region. The amino acid composition and the main chemical structure for various fibroins is summarized in Table 1 [4]. More than 75% of the amino acid composition of *B. mori* fibroin consists of glycine, alanine and serine. The crystalline region of *B. mori* fibroin has a chemical repeat of the type (Gly–X), where *X* stands for *L*-alanine and *L*-serine in a 2/1 ra-

tio [5]. The liquid silk, produced by wild silkworm, e.g. *Antheraea pernyi*, has a molecular constitution that is very close to the homopolymer of poly(*L*-alanine). The main chemical structure of the wild silk fibroin, which contains serine and tyrosine, increases the silk's hydrophilicity thereby allowing it to readily dissolve in water. However, the corresponding homopolypeptides or statistical polypeptides of glycine and *L*-alanine are only soluble in strong solvents such as trifluoroacetic acid, dichloroacetic acid, etc. [6–7]. The amino acid sequence of polypeptides plays a very important role in the solubility and crystallization of silk fibroins.

The results of studies on the growth of spherulite of *Antheraea pernyi* [8–10] and *Antheraea yamamai* silk fibroins, show that large-scale spherulites can form easily, in contrary to poly-(*L*-alanine) spherulites. However, spherulites of *B. mori* fibroin are more difficult to grow [11]. Crystallization of the liquid silk from wild silkworms occurs readily due to heat and not to shear stress. On the other hand, crystallization of *B. mori* liquid silk results from shear stress and dehydration, but not to heat.

Past researchers have investigated the thermal properties of films prepared by drying liquid silk of *Bombyx mori* and *Antheraea pernyi* under fixed drying conditions and films obtained from various concentrations of liquid silk dispersed in water [12–16]. However, there are no detailed papers of the thermal behavior of liquid silk for either domestic or wild silkworms. In the present report, the thermal properties of various liquid silks are investigated by differential scanning calorimetry (DSC). The differences between the characteristic behavior of domestic and wild silkworm liquid silk are discussed based on their thermal properties.

Experimental methods and materials

Liquid silks from five different species of silkworms were analyzed: the domestic silkworm, *Bombyx mori* and four varieties of wild silkworms, the Eri silkworm (*Samia cynthia ricini*), the Japanese giant silkworm (*Dictyoploca japonica*), the Chinese tussah silkworm (*Antheraea pernyi*) and the Japanese tussah silkworm (*Antheraea yamamai*). The liquid silk was extracted from the silk glands of mature silkworms. Each silk gland is divided into three divisions, the Anterior (Ad), the Middle (Md) and the Posterior divi-

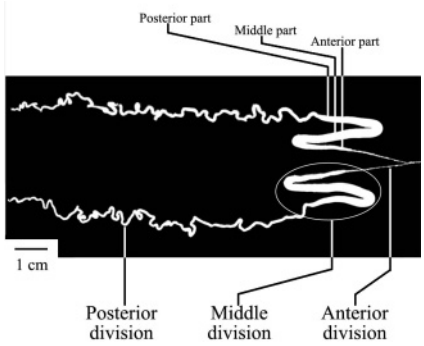

Fig. 1 Photomicrograph of the silk gland of the domestic silkworm, *B. mori*; (Anterior part in Middle division: MA, Middle part in Middle division: MM, Posterior part in Middle division: MP)

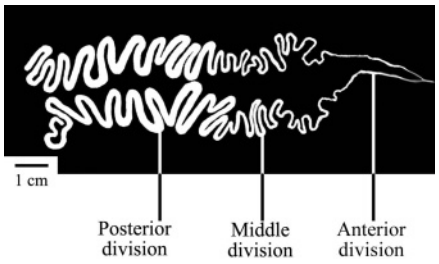

Fig. 2 Photomicrograph of the silk gland of the wild silkworm, *Antheraea pernyi*; (Anterior division: Ad, Middle division: Md, Posterior division: Pd)

sion (Pd), prior to reaching the spinneret region (Figs 1 and 2). For the domestic silkworm, three sections were obtained from the Middle division of the silk gland: the Anterior part (MA), Middle part (MM) and Posterior part (MP) (Fig. 1). For the wild silkworm species, two parts were obtained from the silk gland for thermal analysis: the Middle division (Md) and the Posterior division (Pd) (Fig. 2).

Differential scanning calorimetry was performed with a Seiko DSC 6100 instrument. A standard heating rate of 5°C min^{-1} over the temperature range 0 and 120°C was used.

Results and discussion

Liquid silk from Bombyx mori

The DSC curves of the *B. mori* liquid silk are shown in Fig. 3. Silk from each part of the silk gland of domestic silkworm displays a weak endothermic peak around 68°C. The transition peaks of MA, MM and MP were 68.6, 67.8 and 68.2°C. The heat fusions for each section were 0.25, 0.42 and 0.36 J g^{-1}, respectively. Then, the bottom of endothermic peaks of liquid silk was regarded as the transition peaks.

The viscosity of the gel in the liquid silk decreases as it approaches the anterior division [17, 18]. Thus, silk fibroin in the MP and Posterior division is a weak gel

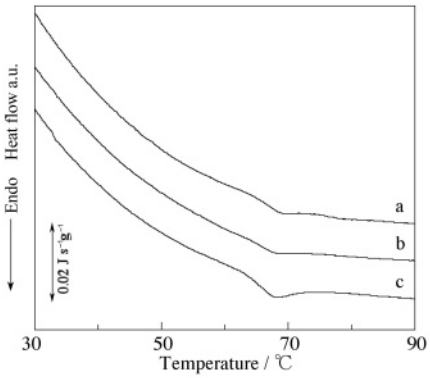

Fig. 3 DSC curves of the liquid silk of the domestic silkworm, *B. mori*; a – MA, b – MM and c – MP

(12 mass%), while the MM is a strong gel (25 mass%), and the MA and Anterior division silk is in a sol state (30 mass%). Fibers are formed as the material is extruded from the spinneret after going through a gel-sol transition in the MA and Anterior divisions. The thermal behavior shown in the DSC curve for the *B. mori* silk supports the gel states discussed above. The heat of fusion shows a high value for the MM section, suggesting that more energy is required to disrupt the silk fibroin when it is in a gel state. MA has a slight orientation towards the fiber axis as it approaches the spinneret region before spinning which would account for higher transition peak temperature among the three middle divisions.

The DSC curves for a second scanning over the same temperature range, 0 and 120°C, show no transition peaks. This shows an irreversible phase transition, or denaturation, and not a glass transition that transferred from glassy state to rubbery state.

Three silk fibroin conformations are known from X-ray diffraction and infrared spectroscopy; random coil, the α-form (silk fibroin I: crankshaft pleated structure or repeated β-turn type II structure), and the β-form (silk fibroin II: anti-parallel chain pleated sheet structure), that can be obtained under various conditions [19–22]. For example, aqueous solutions and films cast from solution have a random-coil conformation. The liquid silk, having a random coil conformation, and then dried at temperatures of 0–40°C has an α-form conformation. However, liquid silk dried at above 50°C, treated with organic solvents, or subjected to shearing has a β-form.

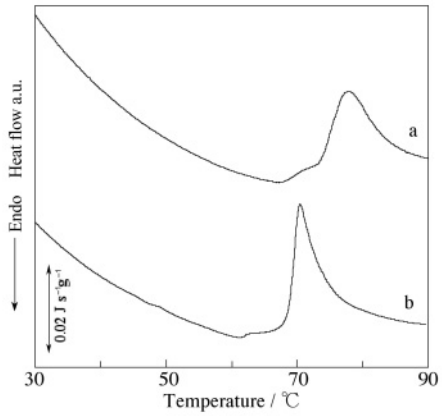

Fig. 4 DSC curves of the liquid silk of the wild silkworm, *Dictyoploca japonica*; a – Md and b – Pd

The DSC curves of samples that were quenched to temperatures between –140°C and 120°C show minute transition peaks, and the clear endothermic peaks around 0°C attributed to the melting of water in the liquid silk. The crystallization of silk fibroin is influenced by the dehydration rate of water in protein: the effect of bound water, not effect of heat treatment. The heat treatment of *B. mori* liquid silk having a random coil conformation destroys some of the structure in the liquid silk. The endothermic peaks observed in the temperature of 0–120°C correspond to the breaking of hydrogen bonds in the α-form crystal structure, or the disentanglement of the physical network present.

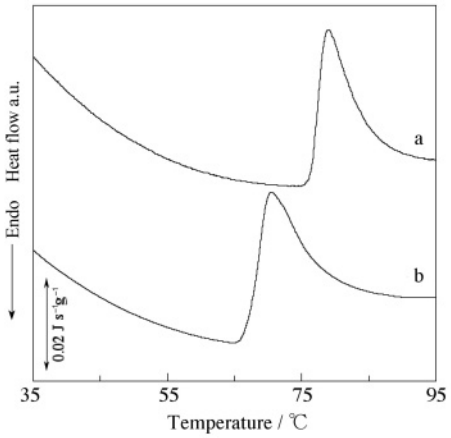

Fig. 5 DSC curves of the liquid silk of the wild silkworm, *Antheraea pernyi*; a – Md and b – Pd

Liquid silk from wild silkworms

The DSC curves of the liquid silk of the wild silkworms; *Samia cynthia ricini*, *Dictyoploca japonica*, *Antheraea pernyi* and *Antheraea yamamai* have a thermal behavior different from that observed for the domestic silkworm. The thermal curves for the liquid silk of *Dictyoploca japonica* and *Antheraea pernyi* are shown in Figs 4 and 5, respectively. The liquid silk from both parts of the silk gland, Md and Pd, from the wild silkworms display clear exothermic peaks for every specimen. The transition peaks and heats of fusion for each liquid silk are summarized in Table 2.

Table 2 Summary of the thermal properties of liquid silk for wild silkworms

	Peak temperature/°C		Heat of fusion/J g^{-1}	
	Middle division	Posterior division	Middle division	Posterior division
Samia cynthia ricini	63.6	59.9	–1.61	–2.50
Dictyoploca japonica	75.8	70.9	–1.77	–2.14
Antheraea pernyi	79.4	69.1	–2.16	–3.24
Antheraea yamamai	85.6	68.4	–1.28	–3.27

Analogous to *B. mori* silk fibroin, the viscosity of the gel of the wild silk fibroin has a tendency to decrease as it moves towards the spinneret region. Thus, silk fibroin in the Pd is a strong gel, whereas that in the Md is a weak gel. The transition from gel to sol is considered to occur at the broader between the Ad and Md. The transition peak of silk fibroin from the Md, occurs at a high temperature than for that in the Pd, indicating that silk fibroin in the Md has a weak orientation before spinning.

The DSC curves for a second scanning over the same temperature range between 0 and 120°C show no transition peaks as for *B. mori*. This indicates an irreversible phase

transition, or crystallization analogous that seen in *B. mori*. Two crystal structures for silk fibroin from wild silkworms, e.g. *Antheraea pernyi*, have been found by X-ray diffraction and infrared spectroscopy, an α-helix and a β-form (or anti-parallel β-sheet) [23, 24]. For example, the conditions required for an α-form containing some random coil to occur in wild silk fibroin include; aqueous solutions, films cast from solutions and liquid silk dried at temperatures below 50°C. Liquid silk dried at temperature above 50°C, or treated with an organic solvent have a β-form.

The DSC curves for samples that have been quenched to temperatures between –140 and 120°C show clear endothermic peaks around 0°C attributed to water melting, and the weak exothermic peaks around 70°C are attributed to the phase transition. The dehydration rate of water in the silk fibroin protein has a little influence on the crystallization. If anything, heat treatment of liquid silk in an α-helix form results in the crystallization into the β-form. The exothermic peaks for synthetic polypeptides, e.g. poly-β-benzyl-*L*-aspartate have been associated with the transition from α-helix to β-form [25]. It is considered that the exothermic peaks in the temperature range of 0–120°C correspond to the phase transition from an α-helix to β-form structure for silk fibroins.

Conclusions

The thermal behavior of the liquid silk for the domestic and wild silkworms summarized in Fig. 6. The DSC curves for the liquid silk from the domestic silkworm have weak endothermic peaks corresponding to the breaking of hydrogen bonds in the α-form or to the untangling of physical network. The DSC curves for the wild silkworm silks, however, show clear exothermic peaks corresponding to a phase transition from the α-helix conformation to the β-form.

The distance from where the protein is synthesized to where silk fiber formation takes place is longer for the wild silkworm than the domesticated *B. mori*. Thus, a

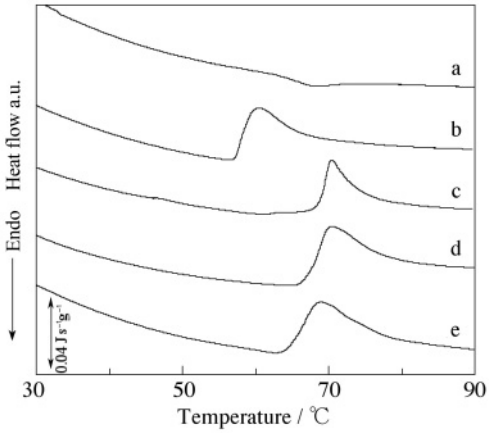

Fig. 6 DSC curves of the liquid silk from the domestic and four wild silkworms; a – *Bombyx mori* (MP), b – *Samia cynthia ricini* (Pd), c – *Dictyoploca japonica* (Pd), d – *Antheraea pernyi* (Pd) and e – *Antheraea yamamai* (Pd)

weak-gel state is maintained for a longer period of time. No fiber could be obtained by directly drawing liquid silk from the silk gland of the wild silkworm without treatment with acid or alcohol. It is more difficult to produce a fiber from liquid silk from the wild silkworm due to the lower shear stresses as compared with those determined for the domestic silkworm. The heat treatment for the wild silk fibroin has strong influence on the crystallization.

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