波 谱 学 杂 志
第 26 卷第 1 期 Vol. 26 No. 1
2009 年 3 月 Chinese Journal of Magnetic Resonance Mar. 2009

**Article**: 1000-4556(2009)01-0017-10

# Temperature-induced Phase Transitions in the Silk Gland of *Bombyx mori* Silkworm Lumen: A High-resolution Solid-state $^{13}C$ NMR study

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**Abstract**: In this contribution, the temperature-induced phase changes of the silk gland of *Bombyx mori* silkworm were studied using high-resolution $^{13}C$ solid-state NMR spectroscopy. The silk gland samples were obtained from alive silkworms which were stored under either normal temperature or 6 ℃ for 2 weeks. The results showed that storing the alive silkworms at 6 ℃ could induce a phase transition in the fibroin gland in the middle and anterior parts of the middle division of the lumen, from an isotropic phase to an anisotropic liquid crystalline phase. Such phase transition was not observed in the silkworms stored under normal temperature. Previous studies have demonstrated, both experimentally and theoretically, lowering temperature can induce an action equivalent to a shearing stress in many elastic polymers (including proteins), and shearing stress may play an important role in the spinning of the silkworm. The results of this study suggest that lowering temperature or applying strong shearing may be used as ways to control the process to fabricate silk-like fibers of high performance.

**Key words**: solid-state NMR, silk fibroin, phase transition, temperature
**CLC number**: O482.53 **Document code**: A

## Introduction

Much attention has been paid to the spinning process of *Bombyx mori* silkworms

**Received date**: 15 Oct. 2008; **Revised date**: 23 Nov. 2008
**Foundation item**: National Natural Science Foundation of China (20434010, 10475017, 20673022).
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under ambient temperature, normal pressure, and with water as solvent, which leads to the production of natural insoluble silk fibers with exceptional mechanical properties[1-9]. It is amazing how the silkworm can stably keep its highly concentrated and stress-sensitive aqueous fibroin in its lumen without the formation of insoluble structures before spinning. Although some characterization for the liquid fibroin stored in the silk gland has been reported, the interesting spinning mechanism of silkworm is still not well understood[5, 10-16].

The sequence of the heavy chain for silk fibroin of *Bombyx mori* silkworm has been determined recently by Zhou[17]. This structural protein is largely composed of repetitions of the motif $(Gly\text{-}Ala\text{-}Gly\text{-}Ala\text{-}Gly\text{-}Ser)_n$. Like the general synthetic polymers and biopolymers, the stereo regularity and orientation of these repeated amino acid motifs could determine the functional properties of these structural biopolymers[18, 19]. There are usually 3 conformations for silk fibroin of *Bombyx mori* silkworm: random coil, α-form (silk I)[20, 21], and β-form (silk II)[3, 22]. The conformation of silk fibroin in solution is dominated by the random-coil[10], whereas the conformation of silk fiber is dominated by the well-oriented β-form[6]. A liquid crystalline intermediate state has been postulated to explain the process from random coil in aqueous solution to insoluble highly oriented fiber[5, 11, 12]. Magoshi[11] suggested that there exists a nematic liquid crystalline order in the anterior division based on the birefringence observation under the polarized light at 20 ℃. Li[12] also proposed a nematic liquid crystalline phase in the middle part and anterior part of middle division but no liquid crystalline phase in the posterior part of middle division based on the birefringence results. Willcox[5] observed the silk spinning process along the way of a silk gland achieved by cryogenic quenching using transmission electron microscopy (TEM), electron diffraction (ED), and atomic force microscopy (AFM), which demonstrated the presence of a cholesteric liquid crystalline phase with periodically spaced size of 200～600 nm for silk fibroin in the proximal part of duct portion of the silk gland. Vollrath and Knight[23, 24] proposed that the spiders and silkworms could spin the fiber with outstanding mechanical properties in a benign environment because the spinning dope was in liquid crystalline state.

The spinning process of the silkworm accompanies many changes such as in pH[25, 26], fibroin concentration[4], shearing stress[4] and metal ions[25, 27, 28], etc. In this contribution, we focused in detail on the phase behaviors of silk gland in different divisions of the silkworm lumen at ambient as well as low temperatures with the method of high-resolution $^{13}C$ solid-state NMR spectroscopy. It has been demonstrated experimentally and theoretically that for many elastic polymers, decreasing temperature could induce an action equivalent to a shearing stress if the polymer phase diagram had a pattern with an upper critical solution temperature (UCST) and meanwhile the formation of the liquid crystalline polymer was subjected to a nucleation-dependent process[29]. Interestingly, the phase diagram of the viscous silk gland does show a UCST pattern[3] and the

fibroin aggregation process is nucleation-dependent[9]. Therefore, we investigated the temperature influence *in vivo* on the phase behaviors of silk fibroin in the silkworms which were lively stored at a low temperature of 6 ℃, slightly higher than 4 ℃ to avoid the high density effect of water herein. An alternative approach was attempted to produce a shearing stress on the fibroin by lowering-temperature. The result may help us to get insight into the fibroin properties and spinning mechanism of natural silkworm, and it could give us a hint to control the phase behaviors of *in vitro* artificial biopolymer.

## 1 Experimental

### 1.1 Materials and sample preparation

Silk fibroin was obtained from silk glands of fifth instar larvae 6～8 day old domestic *Bombyx mori* silkworms. The epithelial surface was gently removed from the gland which then was immersed in the deionized water for 12 hours to remove sericin layers. 3 sections were separated from the middle division of the silk gland: that is, anterior part (AM, toward the spinneret), middle part (MM) and posterior part (PM) (Fig. 1). In order to study *in vivo* the temperature influence on silk fibroin, the silkworms were kept alive in a refrigerator at a temperature of 6 ℃ for 14 days and then dissected as above. As native fibroin is a kind of highly unstable and shearing-sensitive material, the experiments were performed for several times and consistent results were obtained.

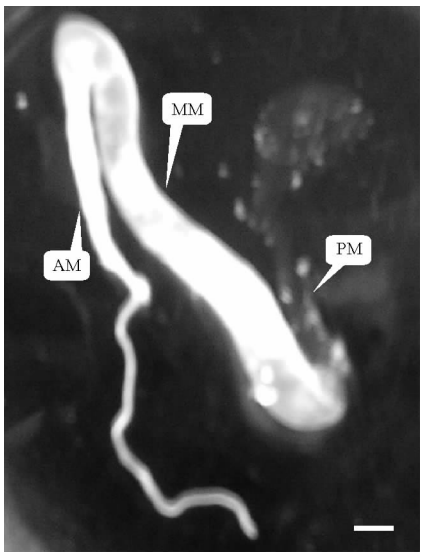


Fig. 1 Image of middle division of the silk gland extracted from a silkworm which was lively stored in a refrigerator at temperature of 6 ℃ for 14 days. The bar in the bottom of the illustration indicates 500 mm

### 1.2 Methods

NMR spectra were recorded on a Varian Infinityplus-300 spectrometer with 7.5 mm double-resonance magic-angle spinning (MAS) probe. Carbon- and proton-field strengths of 35 kHz were applied for the 90° pulses. The field strength was reduced to 15 kHz for proton decoupling. The recycle delays for carbon spectra were set to 10 s for the sufficient relaxation of the nuclear spins. Experiments were carried out at a temperature of 296 K and a magic angle spinning frequency was stabilized at 1 000±5 Hz. The chemical shift was referenced to an external tetramethylsilane (TMS, $\delta=0$).

## 2 Results and Discussion

### 2.1 Phase behavior of silk gland in the middle division of the lumen

The physical state of middle division of silk gland was transparent gel-like dope with average concentration of silk fibroin ~ 25 wt% at room temperature[14]. One-pulse $^{13}C$ static NMR spectra of AM part of silk gland are shown in Fig. 2(a) (without proton decoupling) and Fig. 2(b) (with proton decoupling), and the one-pulse $^{13}C$ MAS NMR spectrum with proton decoupling is shown in Fig. 2(c). Assignments of the spectra are summarized in Table 1 based on the literature[10]. The broad line-shape from $\delta$ 100 to $\delta$ 120 in Fig. 2(a) and 2(b) arises from the chemical shift anisotropic (CSA) of benzene ring of tyrosine residues in the silk fibroin. As seen in Fig. 2, the line widths of the proton-decoupled $^{13}C$ NMR spectrum in Fig. 2(b) are significantly reduced in Fig. 2(c) after the application of magic angle spinning, and no changes in central position of the broad lines away from their isotropic chemical shift are observed, indicating that the residue chemical shift anisotropic and residual dipolar couplings are not observed[30]. The reduction in line width under magic angle spinning results from the averaging of macroscopic susceptibility effects arising from the inhomogeneous sample distribution in the filled rotor rather than residual chemical shift anisotropic and dipolar couplings[31]. We have also checked the effect of spinning rate on the samples from 500 to 1 000 Hz. It demonstrated that only line width was reduced. In general, for a liquid crystal, whose axially asymmetry of $\eta_{CSA}$ is $<1$, one would expect the chemical-shielding anisotropy (CSA), in a static sample, to lead to an inhomogeneous line-broadening and a change in the peak position away from the isotropic shift[31]. Therefore, our results indicate that AM part of silk gland is an isotropic liquid solution rather than an ordered liquid crystal within such a domain size that NMR can observe. The same results for MM and PM parts were observed as well (data not shown). It has been determined by Asakura that the mean correlation time ($\tau_c$) of the segmental motion of silk fibroin in the middle division is in the order of $10^{-10}$ s at 40 ℃[10], that is pertinent to a molecular weight of 500 ~1 000[32] and it means that the corresponding molecular chain domain would be in the

**Table 1 $^{13}C$ chemical shifts ($\delta$) assignment of silk gland[a,b]**

| | $C_\alpha$ | $C_\beta$ | C=O | Others |
|---|---|---|---|---|
| Ala | 50.1 | 16.7 | 175.7[c] | |
| Gly | 42.7 | | 171.6[c] | |
| Ser | -[c] | 61.3 | 172.4[c] | |
| Tyr | 56.0 | 36.2 | 173.7 | 115.7 ($C_\varepsilon$) |
| | | | | 128.1 ($C_\gamma$) |
| | | | | 130.8 ($C_\delta$) |
| | | | | 154.7 ($C_\xi$) |

a. The accuracy of the chemical shift is $\delta$ 0.2; b. The chemical shifts are referenced to TMS; c. Could not be determined unambiguously

range of 10～20 nm if an average molecular weight of each amino acid in silk fibroin were considered being 78[33]. Therefore, we propose that the fibroin in the middle division of silk gland in the silkworm lumen may be in an isotropic liquid phase within the domain of several tens of nanometers, which is equivalent to a random coil polymer in the middle division[10].

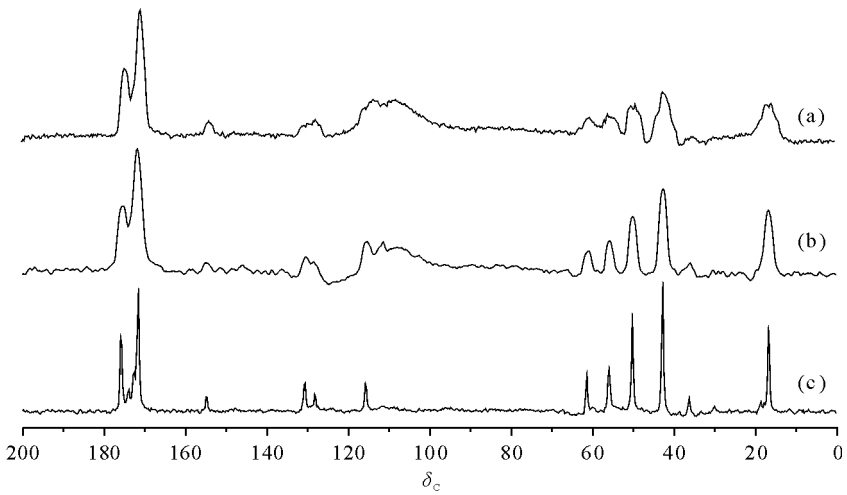


Fig. 2 One-pulse $^{13}C$ NMR spectra of AM part of silk gland. (a) and (b) were reaorded on static samples without and with proton decoupling, respectively; (c) was recorded on a spun sample at spin rate of 1000±5 Hz with proton decoupling

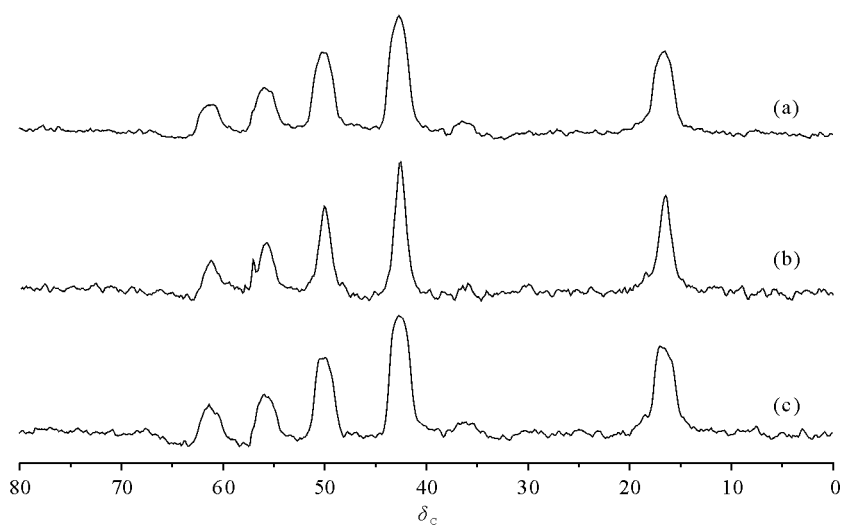


Fig. 3 Aliphatic region of one-pulse $^{13}C$ static NMR spectra with proton decoupling for the parts of PM (a), MM (b) and AM (c) in middle division of the silk gland

However, there are some differences between the spectra of the 3 gland parts of the lumen. The aliphatic region of one-pulse $^{13}C$ static NMR spectra with proton decoupling for the parts of PM, MM and AM are shown in Fig. 3(a), (b), and (c), respectively.

The line widths of the AM part (Fig. 3(c)) and the PM part (Fig. 3(a)) are almost the same, but the line width of the MM part (Fig. 3(b)) is narrower than those of the AM and PM parts. It may indicate that the molecular motion is faster in the wide MM part than in the others.

**2.2 Influence of lowering-temperature on the phase behavior of silk fibroin *in vivo***

In order to observe the temperature influence, the silkworms were kept alive in a refrigerator at the temperature of 6 ℃, slightly higher than 4 ℃ where water has the highest density. It was found surprisingly that, the PM part shown in Fig. 1 remained in liquid state, the AM part looked like an opaque rubber, and the MM part formed a core-shell structure where the core was in a liquid state and the shell was in a rubber-like state. Both the PM and the core of the MM parts treated at low temperature were analyzed by $^{13}C$ NMR using the above techniques (data not shown). And it is also demonstrated that the PM and the MM phase behaviors are in an isotropic liquid phase, which are the same as those of the PM and MM parts at normal temperature. When comparing the $^{13}C$ NMR spectra of low-temperature-treated AM part (Fig. 4(b1) and 4(b2)) with those treated at normal temprature (Fig. 4(a1) and 4(a2)), it is found that the line width of low-temperature-treated rubber-like AM part is broader (Fig. 4(b1)) than that of normal AM part (Fig. 4(a1)), and the peak positions (denoted by dot lines) in Fig. 4(b1) deviate away from the isotropic shifts in Fig. 4(a1), 4(a2) and 4(b2), which indicates that an elastomer of liquid crystal state is formed in the low-temperature-treated AM part. This kind of liquid-crystal state elastomer maybe formed by water compartmentalization and induced by β-sheet formation due to the temperature cooling. The same effect was also observed for the shell of low-temperature-treated MM part (data not shown). The fact that the intensities of serine residue ($\delta$ 61.3) are somewhat higher in the low-temperature-treated samples than those in the normal temperature treated ones is a result of the sericin not being totally removed from the gland. Thus, it is suggested that lowering-temperature does induce the phase transition of the gland *in vivo* from the isotropic phase to the anisotropic liquid crystalline phase, which is similar to a shearing stress effect on the polymer.

Based on the rule of nucleation-dependent aggregation[9], the higher concentration will speed up the proliferation of crystal once the crystalline nucleus is formed. It has been previously measured that the fibroin concentration of posterior division, PM, MM, AM parts in the middle division, and anterior division in the silk gland is about 12%, 12%, 25%, 30%, 30%, respectively[14]. The increase in the fibroin concentration from the PM to the AM parts benefits the formation of liquid crystal through molecular collision, thus, allowing the fibroin to assemble itself into a liquid crystalline phase in the AM part[34] when the shearing force resulted from the stress of the silkworm duct induces the formation of liquid crystalline nucleus. For spontaneous nucleation in static conditions, cylindrical nuclei consisting of the bundle of molecules folded into the length

of 100～200 nm are generally assumed, and theories have been derived on it. Under shearing force, one can expect that the molecules are extended and forced to align parallel. For the silk fibroin, a cholesteric liquid crystalline phase with a length of 200～600 nm was observed in the duct portion of the silk gland[5]. The high content of the repeated hydrophobic units of Gly-Ala-Gly-Ala-Gly- (11% in all 5 263 amino acid residues of heavy chain silk fibroin[17]) makes the formation of the liquid crystal possible as in a synthetic polymer.

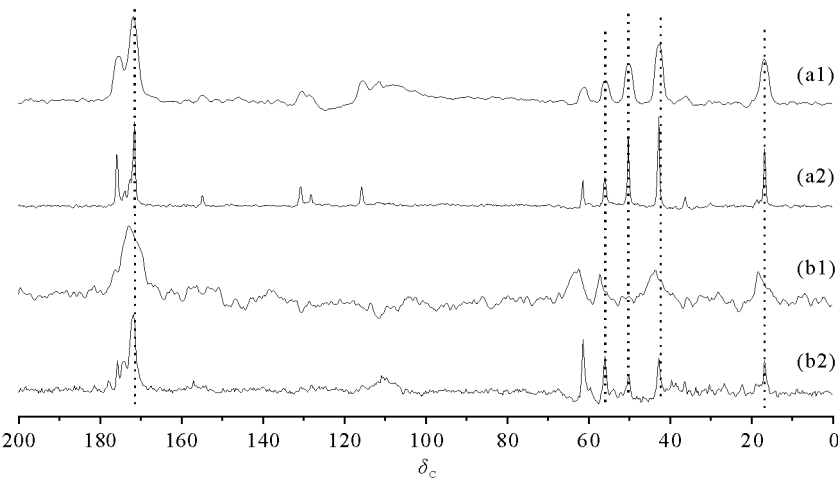


Fig. 4 Comparison between $^{13}C$ NMR spectra of the normal AM part (a1, a2) and those of the low-temperature-treated AM part (b1, b2). (a1) and (b1) are proton-decoupled static spectra, (a2) and (b2) are proton-decoupled MAS spectra at spinning rate of 1 000±5 Hz. The highest peak positions in (b1) (dot lines) deviate away from the isotropic shifts in (b2), indicating that liquid crystalline phase is formed in the low-temperature-treated AM part

### 2.3 Phase behavior model of silk fibroin

Over a domain of several hundreds of nanometers that the usual techniques such as birefringence, TEM, AFM and ED can observe, the phase behavior of silk fibroin in the middle division looks like being in an ordered anisotropic liquid crystal state; however, over a domain of several tens of nanometers, it is in an isotropic state observed by NMR. This type of hierarchical aggregate behavior is actually present in many self-assembled biopolymers[35－37] and synthetic polymers[38], most possibly leading to the formation of liquid crystalline phase when the environmental conditions are proper. During the silkworm spinning, the silk fibroin in the posterior division is in an isotropic liquid phase with a random coil conformation because of its lower concentration herein, but in the anterior division it is likely to aggregate and assemble into an ordered liquid crystalline state in a large molecular motif under a higher fibroin concentration and stronger shearing force in the narrow duct. The gel-like fibroin in the MM part, a wider gland part in the lumen, undergoes a gel-sol transition when passing through the AM part toward to the anterior division, and then the aggregates are extended longer and thinner

due to the strong shearing stress and the water removal close to the spinneret of the silkworm, finally forming a β-sheet conformation. These phase behaviors indicate that highly concentrated viscous silk fibroin could exist stably in the silkworm lumen. In addition, the formation of the self-assembled liquid crystalline phase is possibly facilitated by the metal ions, such as $K^{+}$ [4, 27], $Ca^{2+}$ [25], $Cu^{2+}$ [28] and $Zn^{2+}$ [39] etc.

## 3 Conclusion

The phase behavior of the fibroin in the silk gland is very sensitive to temperature, shearing stress as well as fibroin concentration in the different divisions of silkworm lumen. Normally the fibroin in all the 3 divisions (A, M and P divisions) are present as an isotropic liquid phase. This kind of isotropic liquid state may help the highly concentrated silk fibroin to remain stable in the silkworm lumen. For an efficient spinning, the fibroin in the AM part and the anterior divisions aggregate and assemble into the liquid crystalline phase caused by the shearing stress. Here shearing stress acting on the gland in the lumen *in vivo*, induces a faster aggregation of the fibroin than that without shearing force. The phase transition also occurs in the MM part, possibly preparing the liquid crystal for the AM part. Therefore, we now understand the reason that the spinning process of natural silkworm can undergo at ambient temperature is mainly due to the shearing force. The silkworm spinning process indicates that lowering temperature or strong shearing stress could be used as an approach to control the process to fabricate the silk-like fibers of high performance.

**References:**


[1] Akai H, Imai T, Tsubouchi K. Fine-structural changes of liquid silk in the silk gland during spinning stage of *Bombyx larvae*[J]. J Seric Sci Jpn, 1987, 56(2): 131－137.

[2] Ohgo K, Bagusat F, Asakura T, *et al*. Investigation of structural transition of regenerated silk fibroin aqueous solution by rheo-NMR spectroscopy[J]. J Am Chem Soc, 2008, 130(12): 4 182－4 186.

[3] Kaplan D. Silk Polymers[M]. Washington: American Chemical Society, 1994.

[4] Salamone J C. Polymer Materials Encyclopedia[M]. New York: CRC Press, 1996.

[5] Willcox P J, Gido S P, Muller W, *et al*. Evidence of a cholesteric liquid crystalline phase in natural silk spinning processes[J]. Macromolecules, 1996, 29(15): 5 106－5 110.

[6] Shen Y, Johnson M A, Martin D C. Microstructural characterization of *Bombyx mori* silk fibers[J]. Macromolecules, 1998, 31(25): 8 857－8 864.

[7] Lazo N D, Downing D T. Crystalline regions of *Bombyx mori* silk fibroin may exhibit beta-turn and beta-helix conformations[J]. Macromolecules, 1999, 32(14): 4 700－4 705.

[8] Asakura T, Yamane T, Nakazawa Y, *et al*. Structure of *Bombyx mori* silk fibroin before spinning in solid state studied with wide angle x-ray scattering and $^{13}C$ cross-polarization/magic angle spinning NMR[J]. Biopolymers, 2001, 58(5): 521－525.

[9] Li G Y, Zhou P, Shao Z Z, *et al*. The natural silk spinning process - A nucleation-dependent aggregation mechanism? [J]. Eur J Biochem, 2001, 268(24): 6 600－6 606.

[10] Asakura T, Watanabe Y, Uchida A, *et al*. NMR of silk fibroin. 2. $^{13}C$ NMR study of the chain dynamics and solution structure of *Bombyx mori* silk fibroin[J]. Macromolecules, 1984, 17(5): 1 075—1 081.

[11] Magoshi J, Magoshi Y, Nakamura S. Crystallization, liquid-crystal, and fiber formation of silk fibroin[J]. J Appl Polym Sci: Appl Polym Symp, 1985, 41: 187—204.

[12] Li G, Yu T. Investigation of the liquid-crystal state in silk fibroin[J]. Makromol Chem Rapid Commun, 1989, 10(8): 387—389.

[13] Ochi A, Hossain K S, Magoshi J, *et al*. Rheology and dynamic light scattering of silk fibroin solution extracted from the middle division of *Bombyx mori* silkworm[J]. Biomacromolecules, 2002, 3(6): 1 187—1 196.

[14] Tanaka T, Magoshi J, Magoshi Y, *et al*. Thermal properties of *Bombyx mori* and several wild silkworm silks - Phase transition of liquid silk[J]. J Therm Anal Calorim, 2002, 70(3): 825—832.

[15] Sinsawat A, Putthanarat S, Magoshi Y, *et al*. The crystal modulus of silk (*Bombyx mori*)[J]. Polymer, 2003, 44(3): 909—910.

[16] Hossain K S, Ochi A, Ooyama E, *et al*. Dynamic light scattering of native silk fibroin solution extracted from different parts of the middle division of the silk gland of the *Bombyx mori* silkworm[J]. Biomacromolecules, 2003, 4(2): 350—359.

[17] Zhou C Z, Confalonieri F, Medina N, *et al*. Fine organization of *Bombyx mori* fibroin heavy chain gene[J]. Nucl Acids Res, 2000, 28(12): 2 413—2 419.

[18] Holland C, Terry A E, Porter D, *et al*. Comparing the rheology of native spider and silkworm spinning dope [J]. Nat Mater, 2006, 5(11): 870—874.

[19] Shulha H, Foo C W P, Kaplanb D L, *et al*. Unfolding the multi-length scale domain structure of silk fibroin protein[J]. Polymer, 2006, 47(16): 5 821—5 830.

[20] Lotz B,Cesari F C. The chemical struture and the crystalline structures of *Bombyx mori* silk fibroin[J]. Biochimie, 1979, 61(2): 205—214.

[21] Asakura T, Ashida J, Yamane T, *et al*. A repeated beta-turn structure in poly(Ala-Gly) as a model for silk I of *Bombyx mori* silk fibroin studied with two-dimensional spin-diffusion NMR under off magic angle spinning and rotational echo double resonance[J]. J Mol Biol, 2001, 306(2): 291—305.

[22] Marsh R E, Corey R B, Pauling L. An investigation of the structure of silk fibroin[J]. Biochim Biophys Acta, 1955, 16(1): 1—34.

[23] Vollrath F, Knight D P. Liquid crystalline spinning of spider silk[J]. Nature, 2001, 410(6 828): 541—548.

[24] Knight D P, Vollrath F. Biological liquid crystal elastomers[J]. Philos Trans R Soc Lond Ser B-Biol Sci, 2002, 357(1 418): 155—163.

[25] Zhou P, Xie X, Knight D P, *et al*. Effects of pH and calcium ions on the conformational transitions in silk fibroin using 2D Raman correlation spectroscopy and C-13 solid-state NMR[J]. Biochemistry, 2004, 43(35): 11 302—11 311.

[26] Hu B W, Zhou P, Noda I, *et al*. Generalized two-dimensional correlation analysis of NMR and raman spectra for structural evolution characterizations of silk fibroin[J]. J Phys Chem B, 2006, 110(36): 18 046—18 051.

[27] Ruan Q X, Zhou P, Hu B W, *et al*. An investigation into the effect of potassium ions on the folding of silk fibroin studied by generalized two-dimensional NMR-NMR correlation and Raman spectroscopy[J], FEBS J, 2008, 275(2): 219—232.

[28] Zong X, Zhou P, Shao Z, *et al*. Effect of pH and Cu(II) on the conformation transitions of silk fibroin based on EPR, NMR and Raman spectroscopy[J]. Biochemistry, 2004, 43(38): 11 932—11 941.

[29] Onuki A. Elastic effects in the phase transition of polymer solutions under shear flow[J]. Phys Rev Lett, 1989, 62(21): 2 472—2 475.

[30] Brunner E. Residual Dipolar Couplings in Protein NMR[J]. Concepts Magn Reson, 2001, 13(4), 238—259.

[31] Hronska M, van Beek J D, Williamson P T F, *et al*. NMR characterization of native liquid spider dragline silk from Nephila edulis[J]. Biomacromolecules, 2004, 5(3): 834—839.

[32] Canetti M, Seves A, Secundo F, *et al*. CD and small angle X-ray scattering of silk fibroin in solution[J]. Biopolymers, 1989, 28(9): 1 613－1 624.

[33] McEwen I J, Watt S W, Viney C. The effect of concentration on the rate of formation of liquid crystal and crystalline phases[J]. Polymer, 2001, 42(16): 6 759－6 764.

[34] Perez-Rigueiro J, Elices M, Plaza G R, *et al*. Similarities and differences in the supramolecular organization of silkworm and spider silk[J]. Macromolecules, 2007, 40(15): 5 360－5 365.

[35] Viney C. Natural silks: archetypal supramolecular assembly of polymer fibres[J]. Supramol Sci, 1997, 4(1－2): 75－81.

[36] Martin R, Farjanel J, Eichenberger D, *et al*. Liquid crystalline ordering of procollagen as a determinant of three-dimensional extracellular matrix architecture[J]. J Mol Biol, 2000, 301(1): 11－17.

[37] Zhou H, Zhang Y. Hierarchical chain model of spider capture silk elasticity[J]. Phys Rev Lett, 2005, 94(2): 28－104.

[38] Warner M, Terentjev E M. Nematic elastomers - A new state of matter? [J]. Prog Polym Sci, 1996, 21(5): 853－891.

[39] Li Gui-yang(李贵阳), Zhou Ping(周平), Sun Yao-jun(孙尧俊), *et al*. The effect of metal ions on the conformation transition of silk fibroin(金属离子导致的丝素蛋白的构象转变)[J]. Chem J Chinese Universities(高等学校化学学报), 2001, 22(5): 860－862.

# 低温诱导桑蚕体内腺体相行为的高分辨$^{13}$C 固体核磁共振研究

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**摘 要：** 为什么蚕在常温常压水溶液条件下即能纺出力学性能优异的蚕丝纤维，一直是科学家们感兴趣的问题. 在过去的几十年，人们曾用多种表征手段，如双折射、扫描电镜、原子力显微镜和电子散射等，在界观尺度下对蚕在吐丝过程中腺体的相行为进行研究. 发现腺体在靠近吐丝口时呈液晶态，并认为这是导致蚕丝力学性能的重要因素. 本文则在分子水平尺度下利用核磁共振方法，对五龄蚕活体在常温和 6 ℃下存储数天后解剖的腺体进行研究. 经对化学位移及其线型的各向异性分析发现，当将体内腺体沿吐丝方向分为 3 部，即后部、中部及靠近吐丝口的前部时，常温下，腺体后部和中后部分子呈无规线团，而腺体中中部、中前部和前部分子呈液晶态. 6 ℃时，中后部分子亦呈液晶态，前部分子排列则各向异性更大，说明更为有序. 这种液晶态呈分形结构，在小于纳米尺度下为无规线团，大于纳米尺度呈有序排列. 这表明，降温过程可使呈无规线团的丝素蛋白分子转变为液晶态，其效果如同蚕在吐丝过程中对其腺体施加的剪切应力. 该结果对于人们探索人工合成高性能类丝素纤维的纺丝工艺和条件将有启发和指导作用.

**关键词：** 固体核磁共振；丝素蛋白；相变；温度影响

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