Pressure-Induced Crystal Memory Effect of Spider Silk Proteins

Hongsen Peng, Shaobing Zhou,* Jing Jiang, Tao Guo, Xiaotong Zheng, and Xiongjun Yu

Key Laboratory of Advanced Technologies of Materials, Ministry of Education, School of Materials Science and Engineering, Southwest Jiaotong University, Chengdu 610031, People's Republic of China

Received: December 29, 2008; Revised Manuscript Received: February 3, 2009

Spider silk is a novel material with high strength and toughness and is lightweight, making it attractive for high-performance fiber and biomedical applications. The amino acid sequence and molecular architecture of spider silk protein are becoming known gradually with science and technology development. However, its relationship of structure and function is a puzzle which attracts materials scientists to unveil it. In this study we investigated the changes of the crystal structure as a result of different processing techniques to spider silk protein. Although the crystallization of biomacromolecules is very difficult and complex, we did observe the crystal of electrospun spider silk protein treated by external high pressure in its solid state. This study also provides us a simple and effective method to prepare protein crystals. It is most important that this is the first time that we found spider silk protein as a result of electrospinning that had a crystallization memory property when it was stimulated by a high pressure, which will further extend the list of its particular properties. This finding will also lead us to study it deeply and widen our research on proteins of other organisms.

Introduction

Shape memory materials are stimulus-responsive materials and have received increasing attention in recent years due to their interesting properties and potential applications. Among them, changes in shape and/or size were often induced by external conditions such as temperature, ionic strength, pH, electromagnetism, solvent compositions, and photoirradiation.[1−5] However, to the best of our knowledge, there have been few investigations on the reversible crystal change of spider silk proteins (spiderion) under an external high pressure. It is well-known that, in the world of natural fibers, spider silk is a particularly promising material. Spider silk as a long recognized fiber is one of the most amazing materials, which has outstanding mechanical properties, such as high breaking energy and exceptional toughness, making it attractive for medical, military, and industrial applications.[6] Materials scientists have long been fascinated by its structure and function relationships. In addition to these general properties, recently, a novel shape memory quality in spider draglines was found, and their unrivaled torsional qualities that stop the spider from twisting and swinging further add to its list of remarkable physical credentials.[7]

This study aimed to investigate the crystal memory property of spiderion with an external high-pressure stimulation. Spider silk protein encodes for polypeptides that contain a variable number of both crystalline polyalanine domains and less crystalline glycine-rich domains.[8,9] Although there is growing knowledge about the amino acid sequence and molecular architecture of spider silk proteins, the actual relationship between mechanical properties and function for the natural protein has not been well understand. The crystallization of biomacromolecules such as proteins and nucleic acids is much more difficult and complex than that of small molecules. The reason is that there exist interactions between intra/intermolecular bonds of biomacromolecules due to their high molecular weight, complicated molecular architecture, and surface charge. Protein molecules containing different ionic and polar groups have been crystallized under very different conditions, including variations in ionic strength,[10] pH,[11] high pressure,[12] and an external electric field.[13] However, the present research on protein crystallization mainly focused on silk fibroin, the bacterial protein and lysozyme in a solution state. In this study, spider silk proteins were selected and electrospun to fibers blended with biodegradable poly-D,L-lactide (PDLLA) polymer. The original aim of preparing the composite fibers was to improve the hydrophilicity and biocompatibility of tissue scaffolds by introducing proteins into organic polymers.[14]

Pure spider silk proteins in formic acid solution can hardly be electrospun into fibers, but it becomes easy when they are mixed with PDLLA polymer solution in acetone. In the process, it was accidentally found that the crystallization of regenerated silk proteins showed an obvious difference after the electrospun fibers were treated by a high pressure from X-ray powder diffraction (XRD) analysis. The phenomenon was novel and interesting, so the crystallization of proteins was further investigated in the present study. The crystallization degree of natural spider silk proteins is about 20%, but the crystallization almost disappeared due to the electrospinning process, and finally the crystallization recovered by an external vertical high-pressure stimulation. This is the first time the crystallization memory property of spider silk proteins has been discovered by the evaluation of crystal change with transmission electron microscopy (TEM) and XRD, and the mechanism of crystallization was explained from the conformational changes of the protein performed by analyzing the FT-IR second-derivative spectra and was further illustrated by simulating the conformational changes in the molecular level of proteins.

Experimental Section

Materials. PDLLA (MW 232 000) was synthesized by ring-opening polymerization of D,L-lactide monomer.[15] The molecular weight distribution (PDI) was 1.67 determined by gel permeation

* To whom correspondence should be addressed. Phone: 86-28-87634023. Fax: 86-28-87634649. E-mail: shaobingzhou@swjtu.edu.cn, shaobingzhou@hotmail.com.

10.1021/jp811461b CCC: $40.75
Published on Web 03/18/2009

chromatography (Waters 2695/2414 GPC). Spider silk was produced from Agelena Labyrinthica, which was purified five times in distilled water at room temperature. The so-called names Ss, Ss-F, and Ps-F correspond to the purified spider silk, silk protein film prepared by the formic acid solution-cast method, PDLLA/Ss film prepared by casting from the mixed emulsion of PDLLA/acetone solution and 25% spider silk protein/formic acid solution, respectively. The sample Ps-EF was prepared in the same conditions as the Ps-F sample, except in an external electric field with the indicated electrospinning voltage. All other chemical reagents were of reagent grade and were used as received.

Electrospinning. First, the mixed emulsion of PDLLA/acetone solution and spider silk protein/formic acid solution was added via a 5 mL syringe attached to a circular metal capillary. The circular orifice of the capillary had an inner diameter of 0.6 mm. An oblong counter electrode was located about 15 cm from the capillary tip. The flow rate of the solution was controlled within 3.6−5.4 mL/h by a precision pump (Zhejiang University Medical Instrument Co., Hangzhou, China) to maintain a steady flow from the capillary outlet at room temperature. The applied voltage was controlled within the range of 15−30 kV. Second, the PDLLA/Ss synthetic fabric with a diameter in the range of 200 and 800 nm was obtained with an Ss weight ratio of 25%, which was named Ps. Finally, the nonwoven fiber mats were dried under vacuum at room temperature for 10 h to completely remove solvent residue and stored at 4 °C.

High-Pressure Processing. The regenerated samples from Ss and Ps-F samples pressed by an external high pressure of 20 MPa and Ps sample treated by pressures of 10, 20, and 30 MPa for 2 h at room temperature were named Ss-20, Ps-F-20, Ps-10, Ps-20, and Ps-30, respectively.

Characterization. The samples for TEM (Hitachi H-700H) observation were prepared by directly depositing the as-spun fibers onto the copper mesh. It was operated at 150 k eV.

The IR spectra were obtained with a Nicolet Magna 550 spectrometer equipped with a DTGS KBr detector. For dry samples, 0.8 mg of the sample was mixed with 110−120 mg of KBr and annealed into disks. This process has previously been shown not to alter the IR spectra of dried proteins.[16]

The determination of the secondary structure of proteins was done with a method which combined the second derivative, deconvolution, and band fitting. In the present work, quantitative information was obtained from the deconvolution of the amide I band by following a previously reported procedure.[16−18]

The data and positions of the peaks were taken from the derivative and the deconvoluted spectra. The compositions of the secondary structure could be obtained by fitting the deconvoluted spectrum with Gaussian curves. The area of the different fitting peaks can be associated with different types of the secondary structure.

Crystalline characterization of samples was identified and compared by XL-30 XRD (Philips X' Pert Pro, The Netherlands). The diffractometer with Cu Kα radiation ($\lambda = 1.5406$ Å) from the copper anode source was employed at 40 kV and 40 mA at diffraction angles (2θ) between 10° and 50° performing a step size of 0.03° (2θ) per second.

Results and Discussion

XRD Analysis. Although the composition of the spider silk is not fully known, it has been demonstrated that two types of crystal forms exist in its protein: spiderion 1 and spiderion 2.[19] Spiderion 2 has a hydrogen-bonded antiparallel β-sheet crystal structure which consists of hydrophobic polyalanine domains, whereas spiderion 1, which is composed of less hydrophobic glycine-rich domains, does not display a simple α-helix structure but exists in a state between α-helix and β-sheet. Thus, the perfect crystal of spider silk is hardly obtained and characterized due to its instability of the crystal lattice.

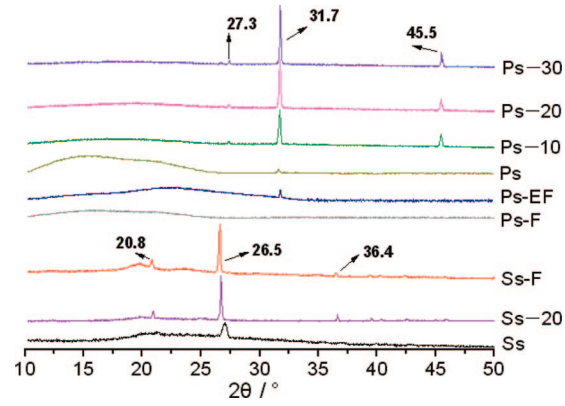

Figure 1. XRD patterns of all samples.

XRD analysis is an excellent method to investigate crystalline materials. As seen from the XRD patterns (Figure 1), compared with the crystals of original Ss, the crystals of all treated samples took changes in crystallinity and in crystal orientation. First, it was found that formic acid as the solvent had an effect on the crystals of protein by comparing the peak of Ss with that of Ss-F. The crystallinity of proteins in Ss-F increased obviously after the proteins recrystallized from formic acid. Second, the electric field with a high voltage of 12−30 kV could influence the crystals of proteins. It decreased obviously after the silk proteins were processed by electrospinning. Since Ss in formic acid solution is very difficult to electrospin, a simple and efficient method is to mix it with PDLLA polymer/acetone solution. The preparation and characterization of an electrospun spider silk fibroin/PDLLA composite fiber have been reported in our previous paper.[14] Therefore, the crystallinity of silk proteins may also be influenced by other factors such as the PDLLA polymer and acetone solvent. to confirm whether the two factors had effects on the crystals, the Ps-F sample was also studied. As seen from Figure 1, its crystallinity almost disappeared after silk proteins were mixed with PDLLA/acetone solution. Therefore, it was deduced that the polymer and solvent could disturb protein crystallization, resulting in a decrease of the crystallinity. To further confirm whether the external electric field with a high voltage could affect protein crystallinity, the sample Ps-EF was prepared by the solution-cast method under it. Comparing the pattern of Ps-EF with that of Ps, it was found that they had a weak crystalline peak at $2\theta = 31.7°$. Therefore, it was concluded that the electric field could bring an effect on the crystal structure. The ionic and polar groups of protein molecules can be rearranged, resulting in a change of the crystal structure when they were stimulated by an electronic field.

Surprisingly, in the final test we found a novel phenomenon that the crystal orientation of all these samples post-treated with an external vertical high pressure except Ps-F had a sharp increase. The crystalline peak of Ss-20 corresponding to the (200) reflection of the original Ss protein became stronger after it was treated with a 20 MPa high pressure. Similarly, the crystalline peak at $2\theta = 31.7°$ also turned stronger and two new peaks at $2\theta = 27.3°$ and 45.5° were presented after Ps was treated with a high pressure. The XRD pattern of the Ps-EF sample was almost identical with that of Ps-10 after it was

TABLE 1: Proportions of the Secondary Structures of Proteins in All Samples

	secondary structure proportions (%)			
sample	β-sheets	random	$\alpha/3_{10}$-helices	turns
Ss	38 ± 2	32 ± 3	13 ± 1	17 ± 2
Ss-20	39 ± 2	14 ± 1	22 ± 2	25 ± 3
Ss-F	60 ± 3	1 ± 1	18 ± 1	21 ± 1
Ps-F	32 ± 1	32 ± 2	17 ± 1	19 ± 2
Ps-EF	39 ± 2	24 ± 1	20 ± 1	17 ± 1
Ps	42 ± 2	23 ± 1	19 ± 2	16 ± 1
Ps-10	46 ± 2	10 ± 1	27 ± 1	17 ± 1
Ps-20	41 ± 1	6 ± 1	24 ± 2	29 ± 2
Ps-30	42 ± 3	6 ± 1	28 ± 2	24 ± 2

treated with the high pressure. However, it was found that the pattern of Ps-F had no changes after it was treated by the same method. The results showed that the high pressure could stimulate the protein crystallization to produce a memory effect and further induce it to grow preferentially for pure Ss and the samples treated with an electric field. After the sample was prepared by mixing the proteins with PDLLA polymer under the nearly same condition without an electric field, its crystallinity did not show any change when it was induced by a high pressure.

Up to now, much of the literature reported that spiderion 1 and spiderion 2 of silk fibroin were studied with X-ray diffractograms. However, spiderion 1 has not yet been studied with the method, and spiderion 2 has been proved by its peaks at 2θ of 16.5°, 20.5°, and 26.5°, which are characteristic peaks of the spiderion 2 (β-sheet) crystallite.[20] Stephens et al. reported that the spiderion 1 crystallite is mainly presented in the electrospun spider silk proteins.[21] By further analyzing the XRD patterns of silk proteins, we can find that the characteristic peaks of the samples, such as Ss, Ss-20, and Ss-F, without being treated with the electric field, have appeared at $2\theta = 20.8°$ and 26.5°, which should also be characteristic peaks of the spiderion 2 form. Moreover, all these samples treated with the electric field had a distinct peak at $2\theta = 31.7°$, which may be a characteristic peak of the spiderion 1 crystallite.

The quantitative analysis of the crystallinity of all samples is shown in Table 1. Comparing the crystallinity of the Ss (15.9%) sample with that of Ss-F (28.1%), it can be seen that the crystallinity of the protein recrystallized from formic acid was enhanced. However, it was nearly lost for samples Ps-F, Ps-EF, and Ps while the protein was mixed with PDLLA/acetone solution, which suggested that the regular arrangement of protein molecular chains was restrained during the solvent evaporation. Moreover, the diffraction peak of the samples as a result of the electrospinning process could not be observed in Figure 1, so the crystallinity of protein was hardly calculated. In the following test, Ps treated with an electric field was further pressed by a vertical high pressure, and then it was found that its crystallinity had a sharp rise. The result indicated that the electric field could make the crystallinity of the protein decrease temporarily but the high pressure could make its crystallization recover.

Analysis of Electron Diffraction of TEM. To further investigate the effect of the processing methods on the protein crystal structure, electron diffraction (ED) of TEM was employed. Figure 2 shows electron diffraction of the proteins in the typical samples of Ss, Ps, Ss-20, and Ps-20. The ED pattern of native proteins (Figure 2, Ss) reveals that the diffraction spots formed diffuse rings, which results from the existence of a polycrystalline structure. [22] Instead, the diffraction spots of Ps are mainly amorphous halos, indicating that its crystallinity is very low, which agrees well with the result of XRD. It also reveals that the external electric field could make the crystallization decrease and even disappear. On the basis of the XRD result that pressure can induce the proteins to take a preferred orientation, we further investigated the effect of the high pressure on the crystal form of protein. By contrast with Ss, the degree of crystal orientation of Ss-20 could be improved after the

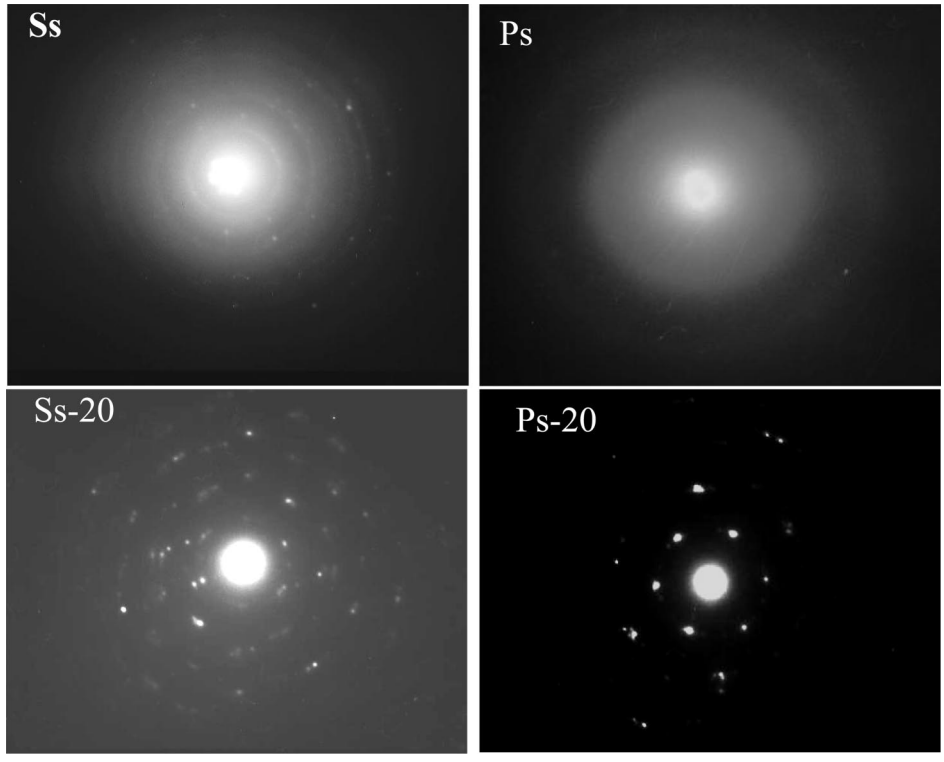

Figure 2. Typical electron diffraction patterns of proteins in Ss, Ps, Ss-20, and Ps-20 samples

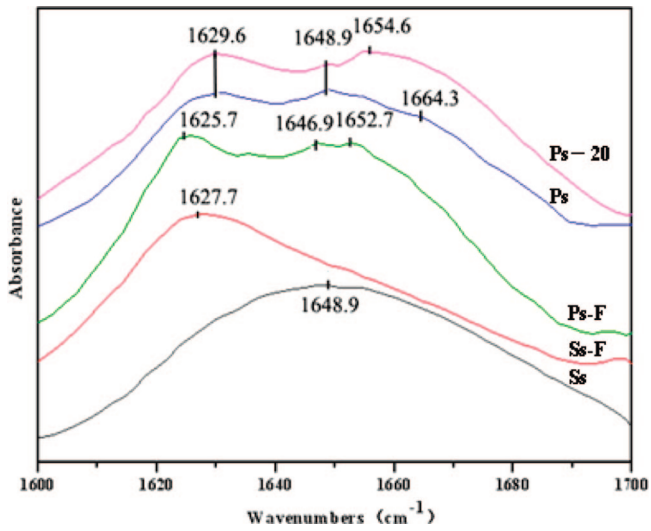

Figure 3. FT-IR spectra in the amide I region of proteins in Ss, Ss-F, Ps-F, Ps, and Ps-20 samples.

protein was high-pressure pressed, and the polycrystalline electron diffraction rings were indicative of an antiparallel β-sheet crystal structure. Similarly, only diffraction spots existed in the TEM photo (Figure 2, Ps-20), indicating that the high pressure could bring a higher molecular orientation to silk protein molecules after they were treated with electrospin processing. The high pressure can promote the crystal form of proteins to transform from polycrystalline to a single crystal in the solid state. On the basis of previous results of the literature[20,23] and the ED photos of Ss-20 and PS-20, we can conclude from the crystal structure that the spiderion 2 and spiderion 1 crystallites belong to trigonal and hexagonal systems, respectively. Therefore, the result further indicated that the high pressure could make the protein crystallization recover, which was consistent with that of XRD analysis.

FT-IR Analysis. The crystal change of the protein is dependent on its molecular structure change. The amide group of proteins and polypeptides presents characteristic vibrational modes (amide modes) which are sensitive to the protein conformation. In our previous literature, it has been reported that the conformational changes of the silk protein were analyzed with the FT-IR second-derivative spectra, from which quantitative information was obtained via the deconvolution of the amide I band.[14] In the present study, to analyze the effect of the electric field and high pressure on the protein conformation, the same method was used to obtain the spectral half-height bandwidth of the single Gaussian components expressed in inverse centimeters and the percentage of each amide I component. Figure 3 shows a comparison of FT-IR absorption spectra in the amide I region of some typical samples. Amide I (1700−1600 cm^{-1} region) is due to the C═O stretching vibration. Amide I bands centered between 1610 and 1641 cm^{-1} are generally considered to be characteristic of β-sheets, a broad infrared band at 1644−1650 cm^{-1} corresponds to a nonordered (random) conformation, and peaks in the range between 1660 and 1652 cm^{-1} are considered to be α-helical structures or other helical structures. The quantitative analyses of the different secondary structures in the protein are shown in Table 1. First, it is found that the used solvent had an effect on the secondary structure of silk protein. By comparing the secondary structure of natural Ss with that of Ss-F, it can be seen that β-sheets increased while the random conformation decreased obviously while the protein was treated with only formic acid, which consisted with the previous report.[21] The random conformation (−31%) of Ss could be converted to β-sheets (+2%), $\alpha/3_{10}$-helices (+5%), and turns (+4%) after it was treated with formic acid solvent. However, the proportion of random conformation (+32%) almost remained stable while a part of the β-sheets (−6%) turned into helices (+4%) and turns (+2%) after natural proteins were mixed with PDLLA polymer and acetone solvent. It was indicated that during the solvent casting process both the PDLLA polymer

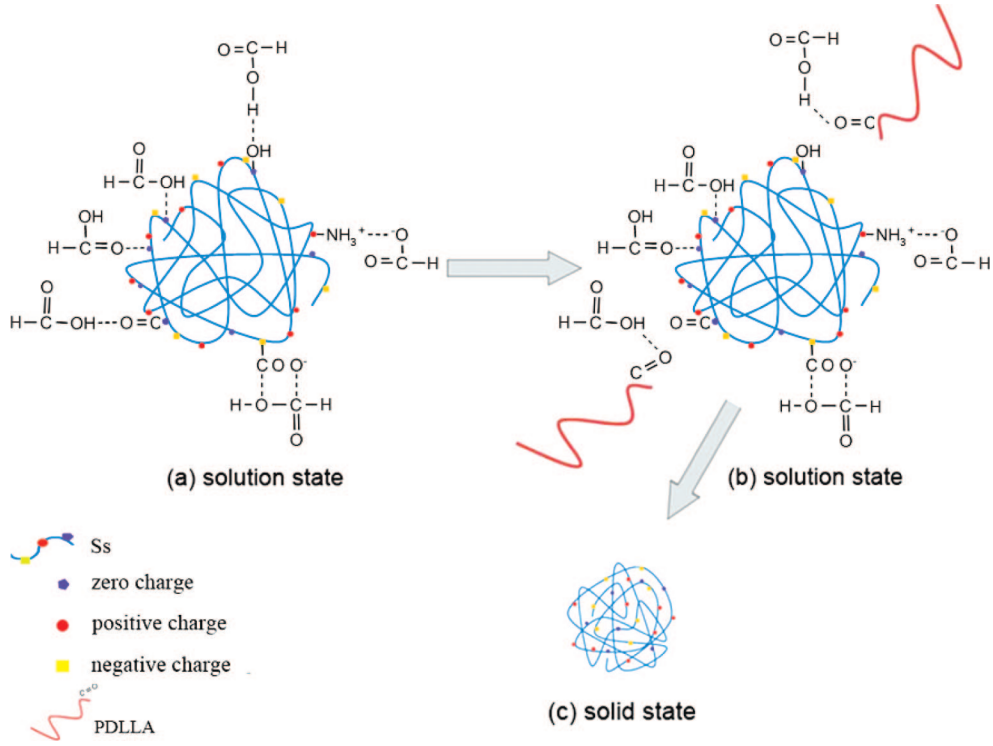

Figure 4. Schematic process of the molecular structure change of protein in the Ps-F sample.

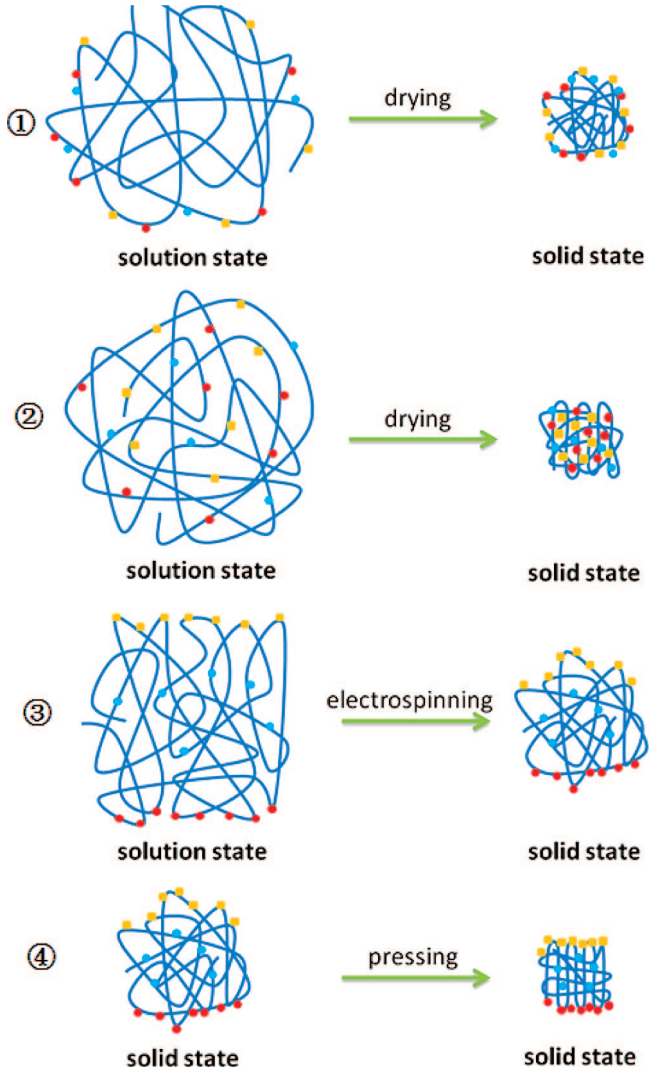

Figure 5. Scheme for the molecular structure changes of proteins processed with different methods in solution and the solid state for (a) Ss-F, (b) Ps-F, (c) Ps, and (d) Ps-20.

and acetone could depress the order of the secondary structure of protein. Second, the electric field with a high voltage could impact the protein conformation. By comparing Ps-F with Ps-EF and Ps, it can seen that the samples treated with an electric field had predominant percentages of β-sheets and helix structures, and their random components decreased evidently. The result suggested that the external electric field has a function of inducing the chain of protein to array more orderly.[21] Finally, it can be observed that the high pressure also influenced the protein conformation. As seen from Table 1, the percentages of β-sheets, $\alpha/3_{10}$-helices, and turns increased for Ss-20, Ps-10, Ps-20, and Ps-30 samples, except the proportion of random conformations decreased acutely. It was further indicated that the molecular chains of proteins in the solid state tended to rearrange from disordered to ordered in the condition of the high-pressure pressing process. Therefore, it was deduced that the change of the secondary structures led to a change of crystallinity for all samples.

Schemes of Protein Molecular Structure Change. Many physicochemical and biological variables can influence the crystallization of biomacromolecules. DNA sequence analysis of different silk genes retrieved from diverse spider species has revealed common protein architectures in their silks. All spider silks show a predominance of glycine, alanine, and glutamine, and there are also substantial amounts of leucine and tyrosine, which are among the larger amino acids.[24,25] The structure of spider silk proteins appears to be much less regular than that of silkworm silk, and spider silks tend to have repeats of Gly-Gly-X (X = Ala, Leu, Pro, Tyr, Glu, and Arg), rather than the Ala-Gly-Ala-Gly-Ser-Gly sequences found in *Bombyx mori* silk.[19,26] The molecular chains of the polypeptide have many carbonyl (CO), hydroxyl (OH), and carboxyl (COOH) groups. Formic acid is a good solvent for spider silk proteins. Since the carboxyl group is composed of a hydroxyl and carbonyl group simultaneously, it can form strong secondary bondings, including hydrogen bonds, with polar groups of other molecules. To explain the above results more clearly and lively, a series of schemes of molecular structure change was designed. As seen from Figure 4a, when the spider silk protein was dissolved in formic acid, the carboxylic groups of formic acid had a potential for interacting with polar side groups of spiderion molecules such as CO, OH, COO^-, and NH_3^+. Consequently, nonpolar side groups assembled much closer in the molecular space, which would give more chance for the protein molecular chains to entangle one another and tend to form a hydrophobic core. The ionic and polar groups of the protein molecules can be rearranged to face outside specifically and thus resulted in their conformational change when the protein solution was dried, as shown in Figure 5a. These behaviors would increase the possibility of local crystallization of protein, resulting in the creation of a β-sheet conformation.[27]

As seen from Figure 4b, we find that the polar side groups of protein molecules will compete for interaction with OH of formic acid and with CO of PDLLA polymer after the protein/formic acid solution is mixed with PDLLA/acetone solution. Moreover, some parts of OH of formic acid could be assigned to interact with CO of PDLLA polymer, and some polar side groups of protein molecules kept their original location as in water solution. The protein molecules exhibited the random coil conformation in solution and maintained the same conformation in the solid state upon drying, as seen from Figure 5b. Thus, the crystallinity of proteins in the Ps-F sample depressed resulted from an irregular array of polypeptide chains. The analysis was in agreement with the result of the FT-IR second-derivative spectra that the percentage of β-sheets of proteins decreased. The scheme further illustrated that acetone solvent and PDLLA polymer could disturb protein crystallization.

It is well-known that there were many amino residues in the side chains of protein molecules with a positive charge in acid solution since H^+ in formic acid can form an electrovalent bond ($-NH_3^+$) with $-NH_2$ as shown in Figure 4b. In an external electric field with a high voltage these amino residues with charges will migrate directionally, thus causing the polypeptide chains to reaggregate to form an orderly structure. The environment of the molecules and their aggregates in the crystallization medium can be changed under the field. Taleb et al. also reported that the external electric field can influence the crystallization of biological macromolecules and promote the formation of crystals with good quality and size.[13]

In this study, it can also be found that the crystallinity of proteins in the Ps-EF sample prepared by the solution-cast method in an electric field was improved. However, in the course of protein transforming from solution to the solid state, polypeptide chains could not be arrayed regularly and tend to form a relaxation structure temporarily due to the very rapid evaporation rate of solvent resulting from the electrospinning process, as shown in Figure 5c. Therefore, its crystallinity measured by XRD is very weak. Figure 5d illustrates that when

the electrospun fibrous film was pressed with an external vertical high pressure, some displacement changes took place in the spatial structure of the protein and the relaxation structure was compacted again in the solid state. The external pressure stimulated the peptide chains to rearray more regularly and closely, which further induced its crystallinity to improve significantly. On the basis of FT-IR analysis of the protein, it was concluded that the pressure could make random conformation decrease sharply and make other secondary structures increase correspondingly. Meanwhile, according to the illustration in Figure 5b, the peptide chains had tangled with each other to form a close core structure, so the pressure had little effect on the crystallinity of Ps-F prepared by slow evaporation of solvent at room temperature.

Conclusions

In summary, proteins in all samples processed with an external electric field were predominantly the spiderion 1 crystallite, and conversely, other samples were spiderion 2 rich structures. Its crystallinity depressed and even disappeared temporarily after the protein was processed by electrospinning, whereas it was returned to a better crystal state after being treated by high pressure as seen from XRD patterns. However, the pressure cannot make the crystallinity of protein in the Ps-F sample recover. Therefore, it can be concluded that the spiderion 1 crystallite played a key role during protein crystal recovery. It could be an important, simple, and effective method to obtain protein crystals with better quality in the solid state. Our results showed that spider silk not only has outstanding tensile strength and toughness but also has a protein crystal memory effect. The crystal memory effect in proteins of other organisms also awaits discovery. Of course, many factors, such as the crystal mechanism, the relationship between crystal memory and the secondary structure, and the reason for producing crystal memory, need to be investigated deeply.

Acknowledgment. This work was partially supported by the National Natural Science Foundation of China (Grant 50773065), Programs for New Century Excellent Talents in University, the Ministry of Education of China (Grant NCET-07-0719), and the Sichuan Prominent Young Talent Program (Grant 08ZQ026-040).

References and Notes

(1) Hu, Z.; Zhang, X.; Li, Y. *Science* **1995**, *269*, 525–527.

(2) Liu, G.; Ding, X.; Cao, Y.; Zheng, Z.; Peng, Y. *Macromolecules* **2004**, *37*, 2228–2232.

(3) Lendlein, A.; Langer, R. *Science* **2002**, *296*, 1673–1676.

(4) Osada, Y.; Matsuda, A. *Nature* **1995**, *376*, 219–220.

(5) Kobatake, S.; Takami, S.; Muto, H.; Ishikawa, T.; Irie, M. *Nature* **2007**, *446*, 778–781.

(6) Vollrath, F.; Knight, D. *Nature* **2001**, *410*, 541–548.

(7) Emile, O.; Floch, A. L.; Vollrath, F. *Nature* **2006**, *440*, 621.

(8) Winkler, S.; Kaplan, D. L. *Rev. Mol. Biotechnol.* **2000**, *74*, 85–93.

(9) Guerette, P.; Ginzinger, D.; Weber, B.; Gosline, J. *Science* **1996**, *272*, 112–115.

(10) Ratanabanangkoon, P.; Gast, A. P. *Langmuir* **2003**, *19*, 1794–1801.

(11) Yatcilla, M. T.; Robertson, C. R.; Gast, A. P. *Langmuir* **1998**, *14*, 497–503.

(12) Suzuki, Y.; Sazaki, G.; Miyashita, S.; Tamura, T. S.; Komatsu, H. *Biochim. Biophys. Acta* **2002**, *1595*, 345–356.

(13) Taleb, M.; Didierjean, C.; Jelsch, C.; Mangeot, J. P.; Capelle, B.; Aubry, A. *J. Cryst. Growth* **1999**, *200*, 575–582.

(14) Zhou, S.; Peng, H.; Yu, X.; Zheng, X.; Cui, W.; Zhang, Z.; Li, X.; Wang, J.; Weng, J.; Jia, W.; Li, F. *J. Phys. Chem B* **2008**, *112* (36), 11209–11216.

(15) Liu, Y.; Xie, M. X.; Kang, J. *Acta Chim. Sin.* **2003**, *61*, 1305–1308.

(16) Yang, T. H.; Dong, A.; Meye, J.; Johnson, O. L.; Cleland, J. L.; Carpenter, J. F. *J. Pharm. Sci.* **1999**, *88*, 161–165.

(17) BÖnisch, H.; Backmann, J.; Kath, T.; Naumann, D.; Schäfer, G. Ü. *Arch. Biochem. Biophys.* **1996**, *333*, 75–84.

(18) Deng, X.; Zhou, S.; Li, X.; Zhao, J.; Yuan, M. *J. Controlled Release* **2001**, *71*, 165–173.

(19) O'Brien, J. P.; Fahnestock, S. R.; Termonia, Y.; Gardner, K. H. *Adv. Mater.* **1998**, *10*, 1185–1195.

(20) Valluzzi, R.; Szela, S.; Avtges, P.; Kirschner, D.; Kaplan, D. *J. Phys. Chem. B* **1999**, *103*, 11382–11392.

(21) Stephens, J. S.; Fahnestock, S. R.; Farmer, R. S.; Kiick, K. L.; Chase, D. B.; Rabolt, J. F. *Biomacromolecules* **2005**, *6*, 1405–1413.

(22) Trancik, J. E.; Czernuszka, J. T.; Cockayne, D. J. H.; Viney, C. *Polymer* **2005**, *46*, 5225–5231.

(23) Valluzzi, R.; Gido, S. P.; Zhang, W. P. *Macromolecules* **1996**, *29*, 8606–8614.

(24) Anderson, S. O. *Comp. Biochem. Physiol.* **1970**, *37*, 705.

(25) Work, R. W.; Young, C. T. *J. Archnol.* **1987**, *15*, 65–80.

(26) Xu, M.; Lewis, R. V. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 7120–7124.

(27) Um, I. C.; Kweon, H. Y.; Lee, K. G.; Park, Y. H. *Int J. Biol. Macromol.* **2003**, *33*, 203–213.

JP811461B