Structural Analysis of Uniaxially Aligned Polymers Using Solid-State ^{15}N NMR

Tetsuo Asakura,*,† Joo-Hong Yeo,† Makoto Demura,† Takuro Itoh,‡ Teruaki Fujito,§ Mamoru Imanari,§ Linda K. Nicholson,⊥ and Timothy A. Cross*,∥

Department of Biotechnology, Faculty of Technology, Tokyo University of Agriculture and Technology, Nakamachi 2-chome, Koganei, Tokyo 184, Japan, R & D, Toyo Seikan Group Company, Hodogaya-ku, Yokohama, Kanagawa 240, Japan, JEOL Ltd., Akishima, Tokyo 196, Japan, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892, and Department of Chemistry and National High Magnetic Field Laboratory, Florida State University, Tallahassee, Florida 32306

Received June 14, 1993

Introduction

It is well known that the mechanical and physical properties of polymers are strongly dependent on the structure of both noncrystalline and crystalline domains in the solid state. However, an atomic level analysis of the polymer structure in the noncrystalline domain is extremely difficult. For example, X-ray diffraction analysis provides only a halo, and hence only limited structural information can be obtained.

The recently developed solid-state ^{13}C and ^{15}N CP NMR methods for the structural analysis of uniaxially aligned proteins and peptides are well suited for the structural analysis of polymers at atomic resolution.[1-4] In solid-state NMR studies of oriented samples, orientation-dependent nuclear spin interaction tensors serve as probes with which the relative orientations of specific bond vectors can be determined. A quantitative analytical approach has been developed to utilize distinctive features of the chemical shifts and line shapes obtained from oriented protein fibers placed parallel and perpendicular to the applied magnetic field.[5] Such data restrict the number of possible orientations of an individual amide plane. This approach has been successfully applied to the structural analysis of silk fibers.[5]

In this paper, we will show a unique graphical representation for the information content of ^{15}N CP NMR spectra obtained from oriented samples placed parallel to the applied magnetic field. In particular, the ^{15}N chemical shifts of the oriented polymers are sensitive to the angle between various molecular fixed axes and the axis of polymer alignment, especially the angle between the NH bond vector and the fiber axis, θ_{NH}. Three kinds of oriented samples whose θ_{NH} values are quite different are chosen here for experimental illustration. The samples include oriented *Bombyx mori* silk fibroin rod in the silk II (antiparallel β sheet) form[5,6] and oriented poly(γ-benzyl L-glutamate) (PBLG) membrane in an α-helical form.[7] In addition, the oriented poly(*p*-phenyleneterephthalamide) (PPTA) fiber is included, whose secondary structure is similar to a β sheet in that the polymer backbone is approximately parallel to the long axis of the fiber.[8]

The structure of poly(*m*-phenyleneisophthalamide) (PMIA) fiber, which contains a considerable amount of noncrystalline domain (about 75–80%),[9,10] will also be analyzed using this polymer structure analysis technique. PMIA has excellent heat stability, although it shows an elastic modulus and tensile strength similar to those of other fibrous polymers.[11] Because of the presence of a large amount of noncrystalline domain in the fiber, the structural analysis of the noncrystalline domain is also included. However, it is extremely difficult to analyze such a disordered structure, and therefore, the structural analysis is limited. Only the crystalline domain in the sample has been analyzed by X-ray diffraction.[12] We show that two distinct ranges of θ_{NH} values are determined for the noncrystalline domain as well as one range of θ_{NH} for the crystalline domain of the PMIA sample.

Theory

The ^{15}N chemical shift anisotropy (CSA) principal axis system (PAS) is the reference frame in which the ^{15}N CSA tensor is diagonal with principal components $\sigma_{11} < \sigma_{22} < \sigma_{33}$.[13] These tensor elements can be determined by observing the powder pattern arising from a randomly dispersed sample. For the case where the fiber (oriented) axis is parallel to the applied magnetic field, the observed chemical shift, $\sigma_{parallel}$, is given by[5]

$$\sigma_{parallel} = \sigma_{11} \sin^2 \beta_F \cos^2 \alpha_F + \sigma_{22} \sin^2 \beta_F \sin^2 \alpha_F + \sigma_{33} \cos^2 \beta_F \quad (1)$$

where α_F and β_F are Euler angles for the transformation from the ^{15}N CSA PAS to the fiber axis system (FAS) fixed in the aligned sample. The chemical shift, $\sigma_{parallel}$, can be calculated as a function of α_F and β_F. On the other hand, the angle θ_{NH} is expressed as follows:[5]

$$\cos \theta_{NH} = \cos \beta_F \cos \beta_{DNH} + \sin \beta_F \cos \alpha_F \cos \alpha_{DNH} \sin \beta_{DNH} + \sin \beta_F \sin \alpha_F \sin \alpha_{DNH} \sin \beta_{DNH} \quad (2)$$

where α_{DNH} and β_{DNH} are Euler angles for the transformation from the ^{15}N CSA PAS to the molecular symmetry axis (MSA) system. When the α_{DNH} and β_{DNH} values are known, θ_{NH} can also be obtained as a function of only α_F and β_F.

Experimental Section

Materials. The highly oriented natural abundance silk fibroin rods (approximately 0.3-mm diameter) in the silk II form were prepared using silk fibroin (concentration 20–30%) from the middle silk gland of the fifth instar stage of the *B. mori* larvae.[14] The gel-like silk fibroin was immersed in dilute acetic acid for a few seconds and then elongated immediately to form the rods. Thin sheets of uniformly aligned silk rods were produced with a quick-setting bonding agent and were cut into 4 × 12 mm pieces, stacked together and fixed with the bonding agent to form a 4 × 4 × 12 mm block which fit within the radio frequency coil of the NMR probe. The silk fibroin powder in the silk II form was prepared as reported previously.[15] The block samples (4 × 4 × 12 mm) of oriented PPTA (Kevlar 49) and PMIA fibers were prepared in a similar manner[5] from materials given by du Pont Japan. The PPTA and PMIA powders were prepared by cutting the fibers. The PBLG powder sample ($M_w = 3.21 \times 10^5$) was a gift from Professor Iizuka of Shinshu University. To prepare the oriented sample of PBLG, 20% (w/v) PBLG solution in dichloromethane (liquid crystalline state)[16] was dropped onto a glass plate and sheared in a single direction by a spatula to form a membrane containing uniformly aligned helices.[17] The solvent was evaporated during this process. The membranes were cut into 4 × 12 mm pieces, stacked together, and bound tightly with Teflon tape to prepare the block sample (4 × 4 × 12 mm) without using a bonding agent. The IR spectrum of the membrane shows that PBLG molecules are in the α-helical conformation.[18] The presence of crystalline domains in silk fibroin rods,[6] PBLG

† Tokyo University of Agriculture and Technology.
‡ Toyo Seikan Group Co.
§ JEOL Ltd.
⊥ National Institutes of Health.
∥ Florida State University.

0024-9297/93/2226-6660$04.00/0

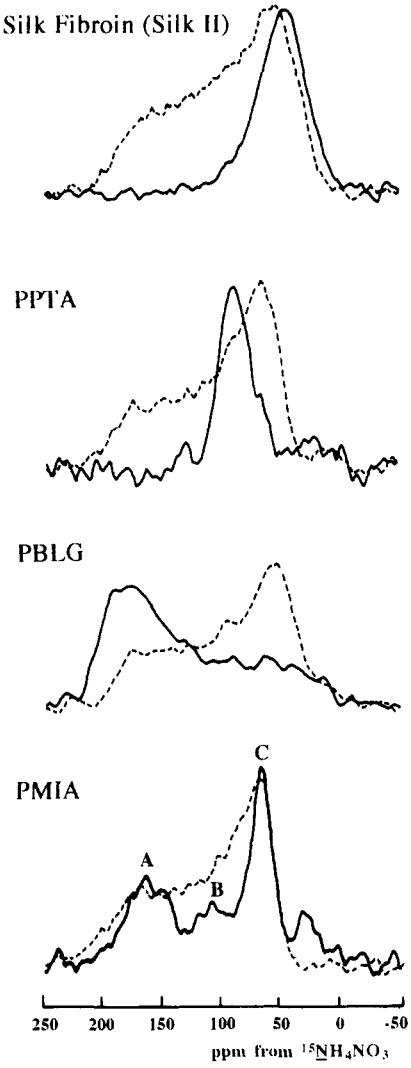

Figure 1. ^{15}N solid-state NMR spectra of the powder (···) and oriented block (- - -) samples of natural abundance *B. mori* silk fibroin, poly(*p*-phenyleneterephthalamide) (PPTA), poly(γ-benzyl L-glutamate) (PBLG), and poly(*m*-phenyleneisophthalamide) (PMIA). The spectra of the oriented block samples were recorded with the fiber axis parallel to the applied magnetic field. The chemical shifts are relative to $^{15}NH_4NO_3$. The spectra were recorded using cross polarization and proton decoupling as described in the text. The numbers of acquisitions are 46 000 (powder) and 15 000 (block) for silk fibroin, 83 000 (powder) and 23 000 (block) for PPTA, 83 000 (powder) and 69 000 (block) for PBLG, and 60 000 (powder) and 48 000 (block) for PMIA.

membranes,[7] PPTA,[8] and PMIA fiber[12] samples were confirmed by characteristic reflections in the X-ray diffraction patterns of these samples.

NMR Measurements. The solid-state ^{15}N NMR experiments were performed at room temperature with a JEOL EX 270 spectrometer operating at 27.25 MHz. Cross polarization was employed for sensitivity enhancement with high-power ^1H decoupling during the signal acquisition period. A 7-μs 90° ^{15}N pulse with a 3-ms mixing time was used. A total of 15000–83000 accumulations with a 7-s repetition time were summed for the data sets as noted in the figure legends.

Results and Discussion

^{15}N CP NMR Spectra. Figure 1 shows the solid-state ^{15}N NMR spectra (solid line) obtained from blocks of oriented natural abundance silk fibroin, PPTA, PBLG, and PMIA samples placed parallel to the applied magnetic field. The small peak at 2–40 ppm in the spectra of the oriented PPTA and PMIA fibers is due to the bonding agent used to prepare the block sample. For the spectrum of oriented silk fibroin, the peak corresponding to the bonding agent is superimposed on the upfield tail of the line shape, and will not significantly affect the value of $\sigma_{parallel}$. The ^{15}N powder pattern spectra (broken line) of the four samples are also shown. The principal components of the ^{15}N chemical shift tensors were determined from simulations of the powder pattern spectra,[13] and are summarized in Table I. For these spectral simulations, a Gaussian line broadening of 10 ppm was applied. Each are homopolymers with only one kind of repeating monomer unit, but the monomer units in PBLG, PPTA, and PMIA are, of course, different from each other. However, silk fibroin is a protein largely composed (about 70%) of a repeating sequence of six residues, (glycine-alanine-glycine-alanine-glycine-serine)$_n$.[14] Therefore, the observed principal components of the chemical shift tensor for natural abundance silk consist of the superposition of the components associated with each amino acid residue having a different chemical shift tensor. The principal components for [^{15}N]Gly-labeled *B. mori* silk fibroin fiber, reported previously, are also listed in Table I.[5] The observed values of the chemical shift tensor elements and $\sigma_{parallel}$ (relative to a fixed chemical shift reference) of the natural abundance silk are larger than the corresponding values of [^{15}N]Gly–silk by 6–10 ppm, but the value of the difference, $\sigma_{parallel} - \sigma_{iso}$, is approximately the same between natural abundance silk and [^{15}N]Gly–silk.

The spectra of the oriented silk fibroin and PPTA polymers placed parallel to the magnetic field yield single peaks with different chemical shifts. The spectrum of PBLG has a tail toward higher field and is different from the former two spectra. The ^{15}N chemical shifts, $\sigma_{parallel}$, for these three polymers are considerably different (Table I). A chemical shift of 48 ppm is observed between σ_{11} and σ_{22} for silk fibroin, a chemical shift of 90 ppm is observed between σ_{22} and σ_{33} for PPTA, and an asymmetric and broad peak with an intensity maximum at 182 ppm, close to σ_{33}, is observed for PBLG. In our previous paper,[5] we reported details of the quantitative analysis of the silk II structure obtained by solid-state ^{15}N NMR of [^{15}N]-Gly-labeled *B. mori* silk fibroin fiber. The spectral pattern is essentially the same as the pattern reported here. The angle between the NH bond and the fiber axis, θ_{NH}, has been determined to be 90° which is the same as that reported by X-ray diffraction.[6,19] The IR spectrum of the PBLG sample used here indicated an α-helical conformation.[18] For an α-helix, the angle between the NH bond and the helix axis is approximately 13°.[20] The helix axes are aligned by shearing stress, and hence the degree of alignment is incomplete as judged by the observed asymmetric line shape with a tail on the high-field side. The secondary structure of PPTA in oriented fiber is similar to that of silk fibroin fiber, with the polymer backbone elongated along the general direction of the fiber axis.[8] The θ_{NH} angle in oriented PPTA fiber is calculated from the coordinate reported in the X-ray diffraction analysis at 66°.[8] The observed values of $\sigma_{parallel} - \sigma_{iso}$ for natural abundance silk fibroin, [^{15}N]Gly-labeled silk fibroin, PPTA, and PBLG are plotted versus θ_{NH} for $0° \leq \theta_{NH} \leq 90°$ in Figure 2. The value of $\sigma_{parallel}$ shifts to higher field by more than 120 ppm upon increasing the angle from 13° to 90°. Hence, the large difference in chemical shifts observed for silk fibroin and PBLG is typical of the difference that would be expected between a β sheet and an α helix, both with their long axes aligned parallel to the magnetic field. The smaller difference in chemical shifts observed for silk fibroin and PPTA illustrates the sensitivity of $\sigma_{parallel}$ to differences between similar secondary structures.

Table I. Experimentally Determined ^{15}N NMR Chemical Shift Tensors of Powder Samples and the Chemical Shifts, $\sigma_{parallel}$, of Oriented Samples Placed Parallel to the Applied Magnetic Field for Natural Abundance Silk Fibroin, Poly(*p*-phenyleneterephthalamide) (PPTA), Poly(γ-benzyl L-glutamate) (PBLG), and Poly(*m*-phenyleneisophthalamide) (PMIA) Along with the Data of [^{15}N]Gly-Labeled *B. mori* Silk Fibroin Reported Previously[5] (ppm Relative to the Reference $^{15}NH_4NO_3$)[a]

sample	chemical shift tensor (ppm) σ_{11}	σ_{22}	σ_{33}	σ_{iso}[b]	$\sigma_{parallel}$ (ppm)	$\sigma_{parallel} - \sigma_{iso}$ (ppm)	θ_{NH} (deg)
silk fibroin	30 ± 3	59 ± 2	196 ± 5	95 ± 4	48	−47	90[c]
[^{15}N]Gly-silk[c]	22	54	186	87	42	−45	90[c]
PPTA	48 ± 3	68 ± 2	195 ± 5	104 ± 4	90	−14	66[d]
PBLG	33 ± 3	56 ± 2	195 ± 5	95 ± 4	182	87	13[e]
PMIA	51 ± 3	66 ± 2	196 ± 5	104 ± 4	68	−36	75,80[f]
					105	1	
					160	56	

[a] The angles, θ_{NH}, between the NH bond and the fiber axis determined by X-ray diffraction are also listed. [b] $\sigma_{iso} = (\sigma_{11} + \sigma_{22} + \sigma_{33})/3$. [c] Reference 5. [d] Reference 8. [e] Reference 20. [f] Reference 12.

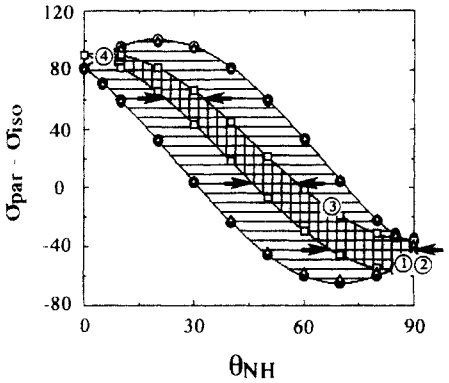

Figure 2. Ranges of $\sigma_{parallel} - \sigma_{iso}$ vs θ_{NH} (0–90°) searched over all α_F and β_F space, shown for α_F and β_F pairs that yield simultaneous solutions to both eqs 1 and 2 in the text. The regions painted by the horizontal lines are for natural abundance silk fibroin (○), [^{15}N]Gly-labeled silk fibroin (●), and PBLG (▲), and the region painted by the vertical lines is for PPTA (□). The θ_{NH} values and the observed $\sigma_{parallel} - \sigma_{iso}$ values for natural abundance silk fibroin (1), [^{15}N]Gly-labeled silk fibroin (2) PPTA (3), and PBLG (4) are included. The three ranges for $\sigma_{parallel} - \sigma_{iso}$ determined from the spectrum of the block PMIA sample are represented by pairs of arrows.

When σ_{DNH} and β_{DNH} values are known, θ_{NH} can be obtained as functions of α_F and β_F using eq 2. The α_{DNH} and β_{DNH} values used here are 0° and 22°, respectively, for silk fibroin and PBLG.[5] For PPTA, 0° and 5° were used for the α_{DNH} and β_{DNH} values which were obtained from the ^{15}N powder pattern spectrum of the model compound, ^{15}N—^{13}C(=O)-double-labeled benzanilide.[21] If a certain θ_{NH} value is assumed in the range of 0–90°, the $\sigma_{parallel} - \sigma_{iso}$ values are obtained by searching all of α_F and β_F space (0–180°) for α_F and β_F pairs that yield simultaneous solutions to both eqs 1 and 2. The orientational restriction for silk fibroins and PBLG is shown by the region painted by the horizontal lines in Figure 2. Similarly, the region painted by the vertical lines is the case for PPTA. The latter region is considerably narrower than the former one, due to the smaller β_{DNH} value. The observed data for silk fibroins (1 and 2), PPTA (3), and PBLG (4) are within these regions. Although the range of possible θ_{NH} values is strongly dependent on the ^{15}N CSA tensor orientation with respect to the molecular frame and can be quite large, the examples above illustrate that $\sigma_{parallel}$ provides a clear distinction between the α helix and β sheet secondary structures.

^{15}N NMR Analysis of Poly(*m*-phenyleneisophthalamide). Using the relationship shown in Figure 2, it is possible to analyze the complex structure of PMIA. Except for the highest field peak (20–40 ppm), which is due to the bonding agent, at least three peaks are observed near 160 (A), 105 (B), and 68 ppm (C), and the approximate peak volume fractions are determined as 39%, 17%, and 44%, respectively. Figure 2 shows that the peaks A, B, and C correspond to the angles θ_{NH} = 25–35°, 46–58°, and 67–90°, respectively. The X-ray diffraction analysis of the crystalline domain of PMIA has been reported by Kakida et al.[12] The values of the angle θ_{NH} were 75° and 80°, which correspond to peak C in the oriented PMIA spectrum. Thus, the ^{15}N nuclei giving rise to peak C are assigned to the crystalline domain, and those giving rise to peaks A and B are assigned to the noncrystalline domain. If the fraction of noncrystalline domain (75–80%) determined from the X-ray diffraction pattern is accurate, then the ^{15}N nuclei in the noncrystalline domain may contribute to peak C. In order to check whether the σ_{iso} value is the same for the nuclei between these two domains, the ^{15}N CP/MAS NMR spectrum of PMIA was observed.[22] The spectrum shows essentially a single peak, indicating that the three peaks must arise from different θ_{NH} values rather than different ^{15}N CSA tensors. If the noncrystalline domain represented a random distribution of θ_{NH} in the oriented PMIA sample, the ^{15}N CP NMR spectrum would be a powder pattern.[13] Clearly, the noncrystalline domains are not composed of randomly dispersed polymer, but exhibit a significant degree of order with remarkably well defined θ_{NH} values. A more quantitative analysis of the PMIA spectra indicating the determinations of θ_{NH} and $\theta_{NC'}$ (angle between the N–C′ bond and the fiber axis) is now in progress.

Acknowledgment. This work was, in part, funded through a Grant-in-Aid of the Ministry of Education, Science, and Culture of Japan (Grant No. 04556007). T.A. gratefully acknowledges support of the Iketani Science and Technology Foundation, Japan, and Yamada Science Foundation, Japan. T.A.C. gratefully acknowledges the support of the National Science Foundation (Grant DMB 90-05938).

References and Notes

(1) Cross, T. A.; Opella, S. J. *J. Mol. Biol.* **1985**, *182*, 367.
(2) Shon, K.-J.; Kim, Y.; Coinago, L. A.; Opella, S. J. *Science* **1991**, *252*, 1303.
(3) Bechinger, B.; Kim, Y.; Chirlian, L. E.; Gesell, J.; Neumann, J.-M.; Montal, M.; Tomich, J.; Zasloff, M.; Opella, S. J. *J. Biomol. MNR* **1991**, *1*, 167.
(4) Ketchem, R. R.; Hu, W.; Cross, T. A. *Science* **1993**, *261*, 1457.
(5) Nicholson, L. K.; Asakura, T.; Demura, M.; Cross, T. A. *Biopolymers* **1993**, *33*, 847.
(6) Marsh, R. E.; Corey, R. B.; Pauling, L. *Biochem. Biophys. Acta* **1955**, *16*, 1.
(7) Elliott, A.; Fraser, R. D. B.; MacRae T. P. *J. Mol. Biol.* **1965**, *11*, 821.

(8) Notholt, M. G. *Eur. Polym. J.* **1974**, *10*, 799.
(9) Kunugi, T.; Murata, T.; Hashimoto, M. *Nihon Kagaku Kaishi* **1975**, 144 (in Japanese).
(10) Kouzai, K.; Matsuda, K.; Tabe, Y.; Honda, H.; Mori, K. *Sen-i Gakkaishi* **1992**, *48*, 55 (in Japanese).
(11) Chatzi, E. G.; Koenig, J. L. *Polym.-Plast. Technol. Eng.* **1987**, *26*, 229.
(12) Kakida, H.; Chatani, Y.; Tadokoro, H. *J. Polym. Sci., Polym. Phys. Ed.* **1976**, *14*, 427.
(13) Mehring, M. *Principles of High Resolution NMR in Solids*, 2nd revd ed.; Springer-Verlag: Berlin, 1983.
(14) Asakura, T.; Watanabe, Y.; Itoh, T. *Macromolecules* **1984**, *17*, 2421.
(15) Asakura, T.; Kuzuhara, A.; Tabeta, R.; Saito, H. *Macromolecules* **1985**, *18*, 1841.
(16) Orwoll, R. D.; Vold, R. L. *J. Am. Chem. Soc.* **1971**, *93*, 5335.
(17) Sasaki, S.; Uematsu, I. *J. Polym. Sci., Polym. Phys. Ed.* **1985**, *23*, 263.
(18) Masuda, Y.; Fukushima, K., Fujii, T.; Miyazawa, T. *Biopolymers* **1969**, *8*, 91.
(19) Takahashi, Y.; Gehoh, M.; Yuzuriha, K. *J. Polym. Sci., Polym. Phys. Ed.* **1991**, *29*, 889.
(20) Fraser, R. D. B.; MacRae, T. P. *Conformations of Fibrous Proteins and Related Synthetic Polypeptides*; Academic Press: New York, 1973; Chapter 9.
(21) Yeo, J.-H.; Demura, M.; Asakura, T.; Fujito, T.; Imanari, M., Nicholson, L. K.; Cross, T. A. Manuscript in preparation.
(22) Asakura, T.; Yeo, J.-H.; Ando, L. Unpublished data.
(23) Hashimoto, M.; Kunugi, T.; Hirai, K.; Zama, H.; Murata, T. *Nihon Kagaku Kaishi* **1974**, 766 (in Japanese).