Diffusional Behavior of Solvent in Polypeptide Liquid Crystalline and Gel States with Highly Oriented Chains As Studied by NMR Spectroscopy

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ABSTRACT: The ^{13}C CP/MAS(cross polarization/magic angle spinning) NMR spectrum of solid poly(γ-benzyl L-glutamate) (PBLG) was measured at the slow spinning rate of 1.9 kHz. The exact principal values of the ^{13}C chemical shift tensor (δ_{11}, δ_{22}, and δ_{33}) for the main-chain carbonyl carbons of PBLG were obtained from an intensity analysis of the spinning sidebands, according to Hertzfeld−Berger method. Further, the ^{13}C CP/MAS NMR spectra and static ^{13}C CP NMR of PBLG were measured in a liquid crystalline solution, where PBLG takes the α-helix form. The experimental results show that there exists a linear relationship between the order parameter S and the observed main-chain carbonyl ^{13}C chemical shift, δ_{obs}, such as $S = 0.024 \times \delta_{obs} - 3.758$. Using the δ_{obs} value and the S-δ_{obs} relation, the order parameter S of PBLG in the liquid crystalline state was determined to be 0.875 ± 0.025. The δ_{obs} value was then used as a monitor for determining the degree of orientation of PBLG in the PBLG gel. Furthermore, the diffusional behavior of 1,4-dioxane as solvent in the PBLG liquid crystalline solution, and in the highly oriented PBLG gel prepared in the magnetic field, was elucidated by the pulsed-field gradient spin−echo ^{1}H NMR method.

1. Introduction

In our previous work,[1,2] we have studied the diffusion behavior of solvents such as chloroform ($CHCl_3$) ("helix" solvent) and trifluoroacetic acid (TFA) ("coil" solvent) on the poly(γ-methyl L-glutamate) (PMLG) gel by using pulse-gradient spin−echo (PGSE) ^{1}H NMR. It is found that the main chain changes from the α-helix to the random coil form by varying the solvent composition of a mixture of TFA and $CHCl_3$. From these experiments, it has been shown that the PMLG gel shrunk transitionally upon changing from the α-helix to the random coil form and that the corresponding diffusion coefficient is transitionally decreased. In the PMLG gel with $CHCl_3$, only the α-helix PMLG chains are randomly cross-linked by a cross-linker. In the gel, the $CHCl_3$ molecules are translating isotropically through the polymer networks.

In addition to the above research work, we have had the motivation to elucidate the nature of the diffusional behavior of a solvent in the directions parallel and perpendicular to α-helical polypeptide chain in polypeptide gel with highly oriented α-helix chains.

As is well-known, PBLG forms the lyotropic liquid crystalline (LC) state in good solvents such as dichloromethane, dioxane, chloroform, and so on,[3−7] and when the PBLG liquid crystalline solution is placed in a strong magnetic field, the main chain can be oriented in the direction of the magnetic field.[8−12] The molecular motion of solvent and PBLG in the liquid crystalline solution is influenced considerably by intermolecular interactions.[17−22] By the reaction of highly oriented PBLG chains with a cross-linker in a strong magnetic field, cross-linked PBLG gel with the highly oriented α-helical chains is expected to be prepared. In the liquid crystalline and the gel states, the orientation of the PBLG chains can be determined by using high-resolution solid-state ^{13}C NMR. As the PBLG chain is undergoing very slow rotation or libration, the ^{13}C NMR spectrum of the polymer cannot be obtained by conventional solution ^{13}C NMR measurements because of the difficulty of the removal of dipolar−dipolar interactions. On the other hand, the diffusion coefficient of molecules with slow motion may be determined by using the pulse-gradient spin−echo ^{1}H NMR method with a strong field gradient.

From such a background, we aim to first prepare cross-linked PBLG gel with highly oriented α-helical chains, to determine the diffusion coefficient of the solvent in the gel for the directions parallel and perpendicular to the α-helical PBLG axis by using the PGSE ^{1}H NMR method with strong field gradient strength as designed by us, and then to elucidate the diffusional behavior of the solvent in the gel. Further, we have determined the degree of orientation of the PBLG chains in the liquid crystalline and gel states from the observation of the ^{13}C chemical shift tensor components of the amide carbonyl carbon of PBLG from solid-state ^{13}C NMR measurements.

2. Experimental Section

2.1. Materials and Gel Preparation. The PBLG (M_w = 130 000) used in this work was supplied by Ajinomoto Co., Ltd. The 25 wt % PBLG lyotropic liquid crystalline solution was prepared with either CH_2Cl_2 or 1,4-dioxane as the solvent. PBLG in the lyotropic liquid crystalline solution was cross-linked with diaminoethane (20−30%/monomer unit) as a cross-linker by the ester−amide exchange reaction to prepare its gel in the magnetic field (6 T) used for the NMR. In this field, PBLG is oriented. When the cross-linker was added to the PBLG solution in an NMR rotor, the NMR rotor was spun for several minutes in the NMR probe, to homogenize the cross-

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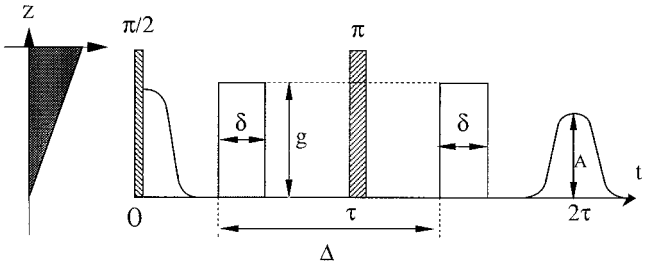

Figure 1. Pulsed-field gradient spin−echo (PGSE) sequence for measuring the diffusion coefficient D.

linker in the PBLG solution. After the start of the cross-linking reaction, the NMR spectrum was recorded as a function of the reaction time to monitor information about the cross-linking during the reaction. Finally, the oriented PBLG gel obtained was swollen in 1,4-dioxane to remove the cross-linker.

2.2. NMR Measurements. Static ^{13}C CP (cross polarization) NMR spectra were measured on a JEOL GSX-270 spectrometer operating at 67.8 MHz and at 300 K. The ^{13}C CP/MAS (magic angle spinning) NMR spectra were measured at the high spinning rate of 4 kHz and at the slow spinning rate of 1.9 kHz. In general, ^{13}C powder pattern spectra are often recorded in order to determine the principal values of the ^{13}C chemical shift tensor. In this work, however, we adopted the spinning-sideband analysis method, that is, the Hertzfeld and Berger method,[13] which allows a determination of the three chemical shift tensor components with high accuracy by using simultaneously all of the information contained in the spinning sidebands as obtained from the slow MAS NMR spectrum. The ^{13}C chemical shift tensor components of the amide carbonyl carbon of PBLG in the solid state then were determined from the slow MAS sideband intensities by using the Hertzfeld and Berger method. The spectra were acquired employing 90° pulses of 4.5 μs duration with proton decoupling. The repetition time was 4.0 s. The liquid crystalline sample was contained in an NMR rotor with a 7 mm o.d. made of zirconia. The contact time was 0.8−2 ms. The spectral width was 27 kHz, and 8000 data points were taken. Spectra were accumulated 500−3000 times to achieve a reasonable signal-to-noise ratio. The ^{13}C chemical shift was determined by using the methine carbon resonance (29.5 ppm) of solid adamantane relative to tetramethylsilane (TMS) as an external reference standard.

The self-diffusion coefficient measurements on 1,4-dioxane as solvent in a PBLG gel were carried out by means of a JEOL GSX-270 NMR spectrometer operating at 270.1 MHz for ^1H with a homemade pulse-gradient generator using a standard PGSE pulse sequence ($\pi/2$ pulse $-\ \tau\ -\ \pi$ pulse) at 298 K, which is the Hahn echo sequence ($\pi/2$ pulse $-\ \tau\ -\ \pi$ pulse), and then two field gradient pulses are added between the $\pi/2$ and π pulses as shown in Figure 1. The field gradient NMR probe was specially designed to generate the strong field gradient pulse (with a maximum field strength of about 22.8 T m^{-1} [2280 G/cm]) and to suppress the eddy currents induced. In this work, a field gradient strength of about 12.8 T m^{-1} [1280 G/cm] was used. The spectral width was 4.0 kHz, and the data points were 4096. The relationship between the echo signal intensity and pulse field gradient parameters is given by

$$A(\delta)/A(0) = \exp[-\gamma^2 G^2 D\delta^2(\Delta - \delta/3)] \qquad (1)$$

where $A(\delta)$ and $A(0)$ are echo signal intensities at $t = 2\tau$ with and without the magnetic field gradient pulse, respectively, whose length is δ. τ is the pulse interval, γ the gyromagnetic ratio of the proton ($\gamma = 2.675 \times 10^{11}$ rad G^{-1} s^{-1}), G the field gradient strength, D the self-diffusion coefficient, and Δ the gradient pulse interval. The echo signal intensity was measured as a function of δ. The plot of $\ln[A(\delta)/A(0)]$ against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ gives a straight line with a slope of $-D$. Therefore, the D value can be determined from this slope. The τ, Δ, and δ values employed in these experiments were 10 ms, 10 ms, and 0.001−0.12 ms, respectively.

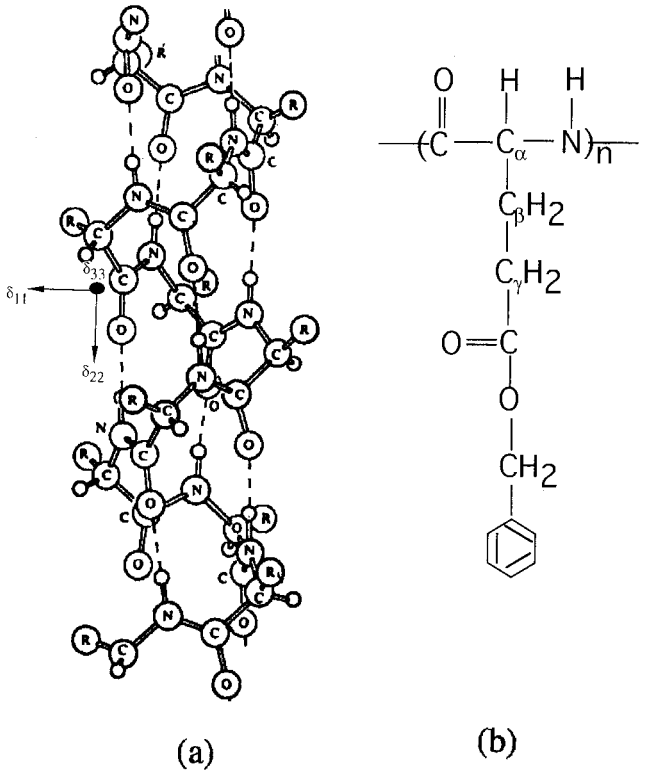

Figure 2. (a) α-Helical structure of a polypeptide chain with hydrogen bonds where R indicates side chain. (b) Chemical structure of the monomer unit of PBLG.

The D value of 2.5×10^{-5}cm^2 s^{-1} for water at 303 K was used to calibrate of the field gradient strength. The experimental error for the D value is estimated to be less than 5%.

3. Analysis of the Orientation of PBLG in the Liquid Crystalline State by the Solid-State ^{13}C NMR Method

For any specified orientation of PBLG, the coordinate system may be oriented such that the magnetic field is not parallel to one of the principal axes in the chemical shift tensor components (δ_{11}, δ_{22}, and δ_{33}). In this case, the chemical shift (δ_{ZZ}) depends on the angles (θ, φ, and ψ) which specify the orientation of the magnetic field direction relative to the principal axes, as well as the absolute magnitudes of the principal values. Thus, the chemical shift (δ_{ZZ}) is expressed by

$$\delta_{ZZ} = \delta_{11}\cos^2\varphi\sin^2\theta + \delta_{22}\sin^2\varphi\sin^2\theta + \delta_{33}\cos^2\theta \qquad (2)$$

In Figure 2, the three-dimensional structure of an α-helical polypeptide chain with hydrogen bonds between the >C═O and H−N< groups is shown together with the chemical structure of PBLG. The hydrogen bond direction is along the α-helical chain. The principal value of δ_{22} is almost directed along the >C═O bond and thus is almost along the α-helical chain axis as shown in Figure 2a. Therefore, for an oriented α-helical polypeptide chain, we have

$$\delta_{ZZ} = \delta_{22} \qquad (3)$$

where $\varphi = 90°$ and $\theta = 90°$. This is a specific case. In fact, the observed chemical shift δ_{obs} becomes δ_{22}, and thus $\delta_{obs} \neq \delta_{ZZ}$ because there exists the angle σ between the α-helical chain axis and the magnetic field direction,

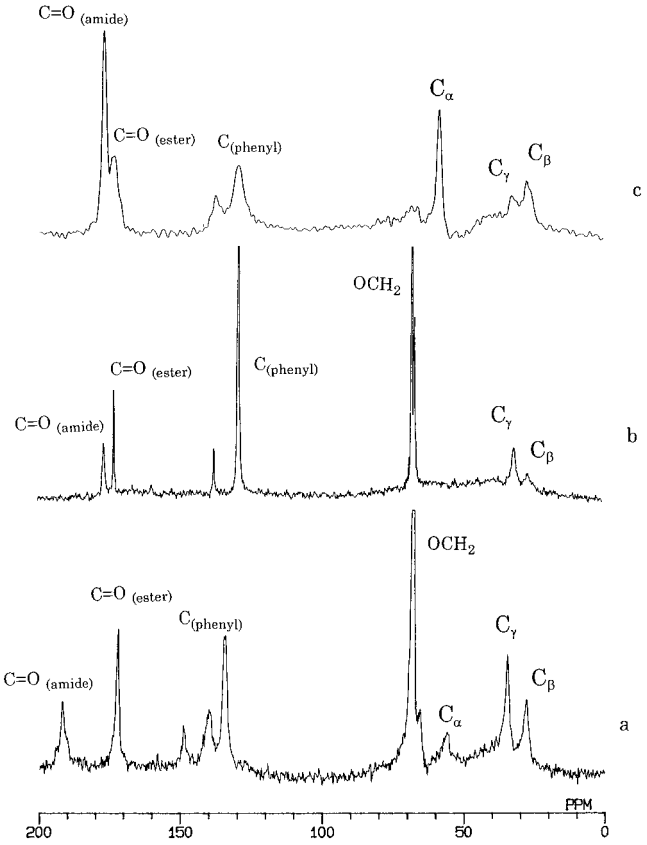

Figure 3. (a) Static ^{13}C CP NMR spectrum and (b) ^{13}C CP/MAS NMR spectrum of a 25 wt % PBLG/dioxane liquid crystalline solution at 67.8 MHz and 300 K and (c) ^{13}C CP/MAS NMR spectrum of PBLG in the solid state at 67.8 MHz at 300 K.

which may take any specified value. In the case of some biomolecules, it is reported that the standard deviation of the angle σ is about 16.85°.[14,15] Therefore, the order parameter S of the α-helical polypeptide chains with respect to the applied magnetic field can be expressed by

$$S = (3\cos^2\sigma - 1)/2 \qquad (4)$$

Under the MAS experiments, the observed chemical shift is obtained as an average of the three chemical shift tensor components as expressed by

$$\delta_{iso} = (\delta_{11} + \delta_{22} + \delta_{33})/3 \qquad (5)$$

4. Results and Discussion

4.1. Spectral Assignments. The static ^{13}C CP NMR spectrum of 25 wt % PBLG/dioxane liquid crystalline solution is shown in Figure 3a. The assignment of the ^{13}C peaks can be made straightforwardly by reference data on solid PBLG.[16]

The amide and ester carbonyl carbons of PBLG appear at 193.2 and 171.8 ppm, respectively. The C_α, C_{phenyl}, C_1(phenyl), C_{OCH2}, C_β, and C_γ carbons appear at 54.3, 145.6, 133.2, 68.8, 34.9, and 27.8 ppm, respectively. The ^{13}C CP/MAS NMR spectrum of PBLG in the solid state is shown in Figure 3c. It is shown that the ^{13}C chemical shift positions of the individual carbons in solid PBLG are very different from those in the LC state. In addition, the ^{13}C CP/MAS NMR spectrum of PBLG in the LC state is shown in Figure 3b. The ^{13}C chemical shift positions of the individual carbons in the LC state are very close to those in the solid state. These chemical shift values are summarized in Table 1, together with the ^{13}C chemical shift values in the LC state as obtained by the static CP method.

Table 1. ^{13}C NMR Chemical Shifts of PBLG in the Solid State and in Liquid Crystalline State in 1,4-Dioxane

	^{13}C NMR chemical shift/ppm							
sample	C═O$_{(amide)}$	C═O$_{(ester)}$	C_α	C_β	C_γ	OCH_2	C_1 (phenyl)	phenyl
solid state								
CP/MAS	176.2	172.4	57.5	26.2	[a]	66.7	[a]	128.9
TOSS	176.3	173.1	57.3	26.5	31.3	[a]	136.7	129.5
liquid crystalline solution state (in 1,4-dioxane)								
CP/MAS	176.3	172.5	[a]	[a]	31.3	67.4	137.3	128.7
CP(static)	191.2	171.7	55.6	27.4	34.3	68.1	[a]	139.3

[a] The chemical shift cannot be read because of overlapping.

The isotropic ^{13}C chemical shift values for the amide carbonyl carbons of PBLG in the solid state (α-helix) and in the LC state are 176.4 and 176.2 ppm, respectively. These chemical shift values are almost the same, and this means that the main chain of PBLG in the LC state takes the α-helical conformation. The ^{13}C signal of the amide carbonyl carbon (C═O$_{amide}$) in the LC state, as determined by static CP method, appears about 17 ppm downfield of the signal than that in the solid state determined by the CP/MAS method. What is the cause of such a shift? The α-helical chain is first aligned in the magnetic field direction and then the hydrogen bonded carbonyl axis is then oriented to the magnetic field direction. The direction of the principal value δ_{22} of the carbonyl carbon is oriented to the carbonyl bond axis. Therefore, the ^{13}C chemical shift of the carbonyl carbon in the LC state is different from the isotropic ^{13}C chemical shift of solid PBLG. This becomes a very important factor in determining the carbon parameter of PBLG in the LC state as described below. To elucidate the orientation of PBLG in the LC state through the observation of the ^{13}C chemical shift of the amide carbonyl carbon, the ^{13}C chemical shift tensor components are needed. For this, the slow MAS experiments were carried out to determine the chemical shift tensor components with high accuracy.

Figure 4 shows the ^{13}C CP/MAS NMR spectrum of 25 wt % PBLG/dioxane LC solution at the slow MAS rate of 1900 Hz, which has the spinning sidebands in the carbonyl and phenyl carbon region. The intensities of the spinning sidebands of the amide-carbonyl carbon were determined by computer fitting. From the Hertzfeld−Berger analysis with the determined intensities of the spinning sidebands (±1, ±2, and ±3) of the amide C═O carbon, the three principal values of the ^{13}C chemical shift tensor were found: $\delta_{11} = 238.7 \pm 1.8$ ppm, $\delta_{22} = 198.5 \pm 1.0$ ppm, and $\delta_{33} = 92.3 \pm 1.8$ ppm. From the experimental results and the computer simulation, it is found that the chemical shift value δ_{iso} of the amide C═O carbon in the LC state is very close to the δ_{22} value. This means that the principal axis of the amide C═O carbon tensor component δ_{22} is almost oriented in the magnetic field direction.

4.2. ^{13}C Chemical Shift of the Amide Carbonyl and the Order Parameter *S* of PBLG in the LC State. As mentioned above, the ^{13}C chemical shift, δ_{obs}, of the amide carbonyl carbon of PBLG in the LC state obtained by the static CP method is 193.2 ppm which is close to the δ_{22} value (198.5 ppm) of solid PBLG. The former appears 5.3 ppm upfield from the latter. If a PBLG chain is completely oriented in the magnetic field

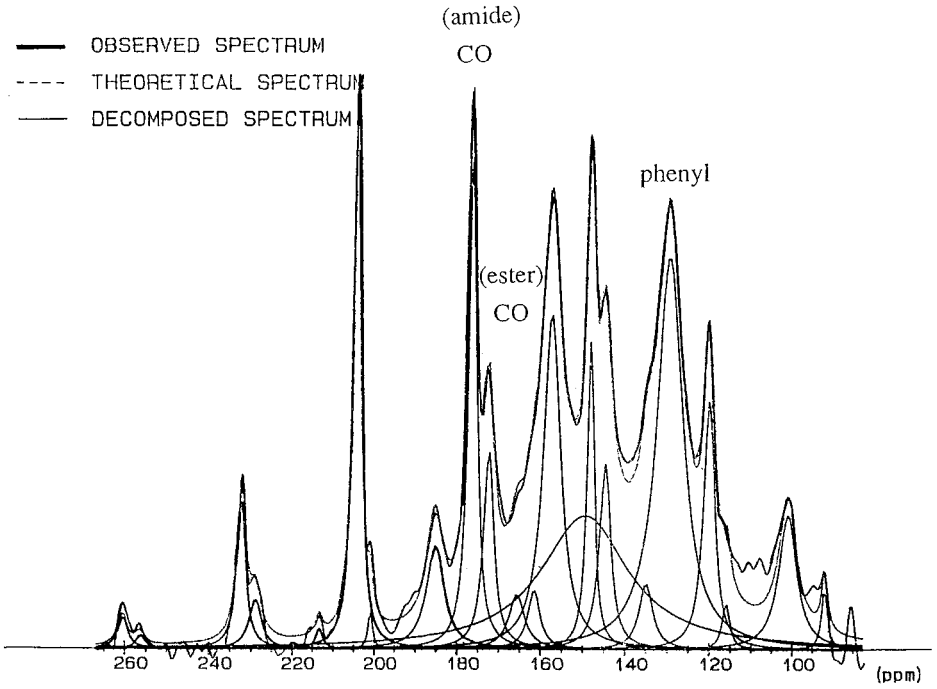

Figure 4. ^{13}C CP/MAS spectrum of 30 wt % PBLG/CH_2Cl_2 liquid crystalline solution at a spinning rate of 1900 Hz (the carbonyl and phenyl carbon region) and the simulated spectrum from the Lorentzian functions.

direction, the direction of δ_{22} agrees with the magnetic field direction, and the order parameter S becomes 1. The observed chemical shift difference (5.3 ppm) between δ_{22} and δ_{obs} shows that a PBLG chain axis is deviated from the magnetic field direction, and thus the direction of the δ_{22} deviates from the magnetic field direction. This shows that the order parameter S becomes smaller than 1 and the PBLG chain is fluctuating around the magnetic field direction.

Suppose that this fluctuation obeys the Gaussian distribution, the x, y, and z coordinates in the molecular system are transformed to the laboratory coordinates. From this situation and eq 2, the chemical shift δ then is expressed by

$$\delta = \int\int \delta(\varphi, \theta) f(\varphi) g(\theta)\, d\varphi\, d\theta$$

$$= \int_{-\pi/2}^{+\pi/2} \int_{-\pi}^{+\pi} \{\delta_{11} \cos^2(\varphi + \varphi_0)\sin^2(\theta + \theta_0) + \delta_{22}\sin^2(\varphi + \varphi_0)\sin^2(\theta + \theta_0) + \delta_{33}\cos^2(\theta + \theta_0)\} \times (1/2\pi\sigma^2) \exp[-(\varphi^2 + \theta^2)/2\sigma^2]\, d\varphi\, d\theta \quad (6)$$

where φ_0 and θ_0 are the average values of the angles φ and θ, respectively, and σ is the standard deviation of Gaussian distribution. By substituting the δ_{obs} into eq 6, we can obtain the relationship between the angles (φ_0 and θ_0) and the standard deviation σ of the Gaussian distribution as shown in Figure 5.

If the α-helical chain axis orients completely in the magnetic field direction, the standard deviation σ becomes 16.85°. This means that the α-helical chain axis is fluctuating around the magnetic field direction with a standard deviation of 16.85°. By substituting this value into eq 4, the order parameter S is found to be 0.875 ± 0.025. For the oriented liquid crystalline PBLG solution system, the relationship between the order parameter S and the value of δ_{obs} can be expressed (as shown in Figure 6) by

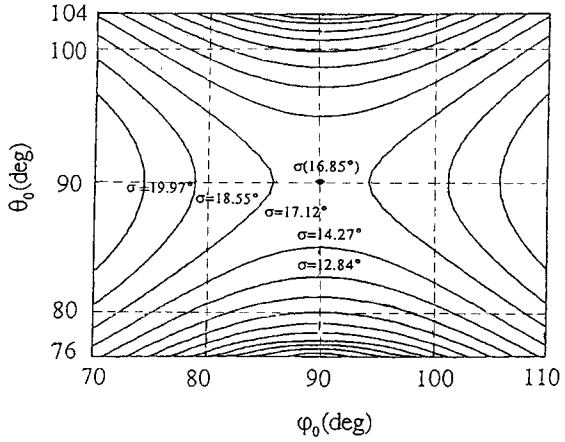

Figure 5. Relationship between the angles (ϕ_0 and θ_0) and the standard deviation σ in a Gaussian distribution (at δ_{obs} = 193 ppm).

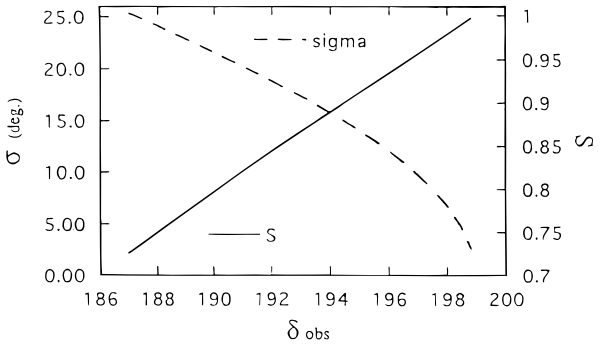

Figure 6. Relationship between δ_{obs} (the observed ^{13}C chemical shift of the main-chain carbonyl carbon in PBLG) and the standard deviation σ in the Gaussian distribution and between δ_{obs} and the order parameter S.

$$S = 0.024 \times \delta_{obs} - 3.758 \quad (7)$$

From this relation, if we obtain the δ_{obs} value, the order parameter S can be determined. In the completely

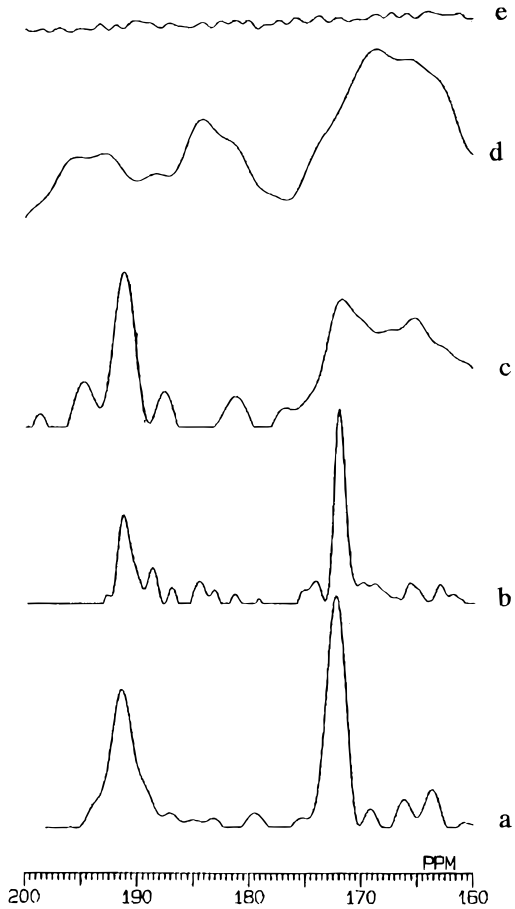

Figure 7. Static ^{13}C CP spectra of the main-chain and side-chain carbonyl carbons in PBLG gel at 25 °C. Diaminohexane is used as a cross-linker. In 25% PBLG/1,4-dioxane liquid crystalline solution system, the reaction times were (a) 0; (b) 1, 0 (c), 3.5 and (d) 14 h in the magnetic field. After the reaction time of 14 h, the system was placed for (e) 20 min outside the magnetic field at 25 °C.

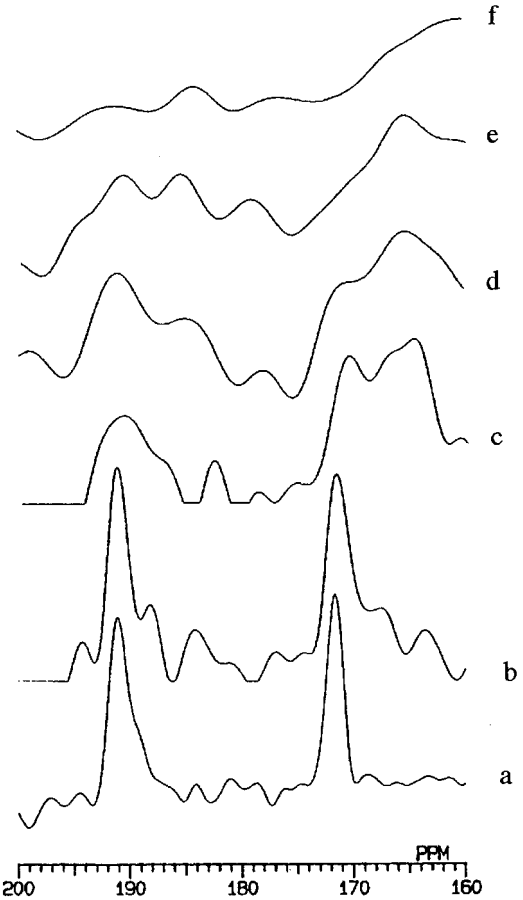

Figure 8. Static ^{13}C CP spectra of the main-chain and side-chain carbonyl carbons in PBLG gel at 25 °C. Diaminoethane is used as a cross-linker. In 25% PBLG/1,4-dioxane liquid crystalline solution, the reaction time were (a) 0, (b) 1, (c) 4, and (d) 18 h in the magnetic field. Spectrum after being placed for (e) 100 h outside the magnetic field. After the reaction time of 18 h, the system was placed for 100 h outside the magnetic field at 25 °C. The obtained cross-linked PBLG sample was swollen in 1,4-dioxane for (f) 50 h.

oriented PBLG system ($S = 1$), $\delta_{obs} = \delta_{22} = 198.5$ ppm. However, in a real system, as the main chain with the α-helical conformation is fluctuating around the magnetic field, δ_{obs} appears upfield from δ_{22}.

4.3. Dependence of the Order Parameters of the Oriented PBLG Chains in the Gel State on the Alkyl Chain Length of the Cross-Linker Used and the Temperature. Static ^{13}C CP spectra of PBLG solutions with 1,6-diaminohexane as cross-linker as a function of the cross-linking reaction time during the PBLG gel formation at 25 °C are shown in Figure 7. As the reaction time is increased, the spectrum becomes broad. When the system was placed for 20 min outside the magnetic field at 25 °C, after the reaction time of 14 h, the signals disappear completely. These phenomena can be explained by the following discussions. The heat generated by the ester−amide exchange reaction leads to an increase in the molecular motion of PBLG. Thus, the CP efficiency for the signal intensity is reduced and the signals disappear gradually. From the ^{13}C chemical shift value of the main chain carbonyl carbon of PBLG in the oriented gel, the degree of orientation for PBLG is greatly reduced. This shows that the orientation of the PBLG chains becomes isotropic.

The static ^{13}C CP spectra of PBLG solution with 1,2-diaminoethane which has a shorter *n*-alkyl chain compared with 1,6-diaminohexane were taken as cross-linker as a function of the cross-linking reaction time during the PBLG gel formation at 25 (Figure 8) and 15 °C (Figure 9) and are shown in Figure 7. The cross-linking reaction was carried out in the magnetic field used for the NMR experiment. As the reaction time is increased, the observed spectrum slowly becomes broad as seen from Figures 8 and 9. After the sample was placed outside the magnetic field for 100 h at 25 °C, the observed spectrum quickly becomes broad as shown in Figure 8. On the other hand, after the sample was placed outside the magnetic field for 100 h at 15 °C, the observed spectrum slowly becomes broad as shown in Figure 9. This shows that at lower temperatures the oriented liquid crystalline structure is not so strongly disturbed by heat generated from the cross-linking reaction. When the cross-linker with a shorter *n*-alkyl chain is used, the oriented liquid crystalline structure is more strongly formed. The observed ^{13}C chemical shift value, δ_{obs}, of the main-chain carbonyl carbon of PBLG in the oriented gel was obtained as a function of the cross-linking reaction time as shown in Figure 10. From this figure, it is seen that the order parameter S is increased with an increase in the cross-linking reaction time at the initial time of the gel formation. However, the order parameter S of the PBLG network chains in the gel decreases with an increase in the cross-linking reaction time after the reaction system was taken from the magnetic field. The order parameter S decreases

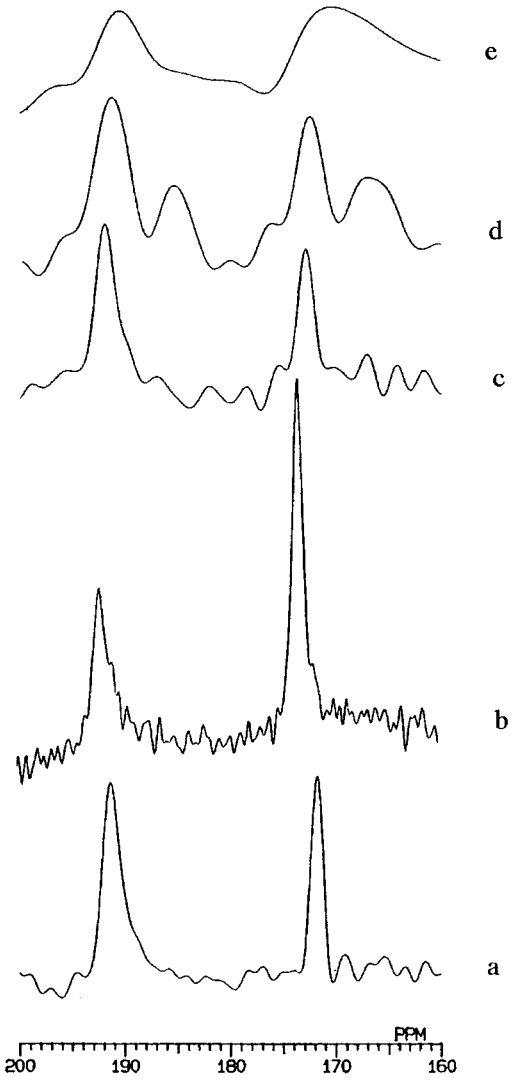

Figure 9. Static ^{13}C CP spectra of the main-chain and side-chain carbonyl carbons in a PBLG gel at 15 °C. Diaminohexane is used as the cross-linker. In 25% PBLG/1,4-dioxane liquid crystalline solution, the reaction times (a) 0, (b) 4, (c) 8, and (d) 18 h in the magnetic field. After the reaction time of 18 h, the system was placed for (e) 100 h outside the magnetic field in a refrigerator with a constant temperature of 15 °C.

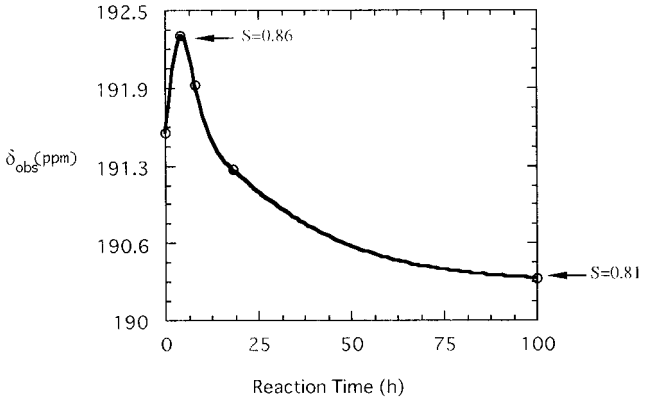

Figure 10. Plots of the observed chemical shifts, δ_{obs} (ppm), of the main-chain carbonyl carbon in a PBLG gel system as a function of the cross-linking reaction time.

from 0.86 to 0.81. As seen from these experiments, reasonable reaction conditions to obtain the oriented polypeptide gel are as follows: a cross-linker with a short *n*-alkyl chain, such as 1,2-diaminoethane, should be used and a lower reaction temperature such as 15 °C should be chosen.

Table 2. Diffusion Coefficients of Dioxane in Various States

	a[a]	*b*[b]	*c*[c]	*d*[d]
D ($\times 10^{-5}$ cm^2 s^{-1})	1.06	0.58	0.54	0.45

[a] 1,4-dioxane in pure liquid. [b] 25% PBLG/dioxane liquid crystalline solution. [c] PBLG gel in the direction parallel to the α-helix axis. [d] PBLG gel in the direction perpendicular to the α-helix axis.

4.4. Diffusional Behavior of Solvent in the Oriented PBLG Gel. The diffusion coefficients of 1,4-dioxane as solvent, contained in the oriented PBLG gel, were determined by the PGSE ^1H NMR method at 303 K. The experiments were carried out under two conditions: one, the PBLG α-helix axis is parallel to the magnetic field, and two, the PBLG α-helix axis is perpendicular to the magnetic field. In our designed PGSE system, we cannot change the direction of the field gradient pulse because we have designed this NMR probe system such that it can generate a very strong field gradient. However, it can be said that during this experiment time (the measurement time of the PGSE method is very short for obtaining the proton signal) the orientations of the PBLG chains in the gel sample do not change with a change of the orientation of the gel sample with respect to the magnetic field. Some crossed polarized microscopy photos of these gels were taken. From these, it is clear that the PBLG chains in the polypeptide gel are highly oriented.

For the peak of 1,4-dioxane in the oriented PBLG gel, plots of $\ln[A(\delta)/A(0)]$ against $\gamma^2 G^2 \delta^2 (\Delta - \delta/3)$ are a straight line (not shown). This shows that the diffusant, 1,4-dioxane, in the gel is a single-component diffusion during the observation time. The diffusion coefficients D of 1,4-dioxane in the oriented PBLG gel under the above-mentioned condition then were determined from the slope of the straight line, and the diffusion coefficients of 1,4-dioxane in the neat liquid and 1,4-dioxane in 25% PBLG/dioxane LC solution were determined. The determined diffusion coefficients D of 1,4-dioxane in the above states are shown in Table 2. The diffusion coefficient of 1,4-dioxane in the neat liquid is 1.06×10^{-5} cm^2 s^{-1} which is much larger than that in the 25 wt % PBLG/dioxane liquid crystalline solution which is 5.8×10^{-6}cm^2 s^{-1}. This means that in the liquid crystalline solution the translational motion of the 1,4-dioxane molecule was strongly restrained by the interaction between the PBLG chains. On the other hand, the diffusion coefficients of 1,4-dioxane in the oriented PBLG gels in which the PBLG chains orient parallel and perpendicular to the applied magnetic field are smaller than that in the PBLG/dioxane LC solution. This means that the translational motion of 1,4-dioxane through the PBLG networks in the gel is restrained much more strongly than that in the LC solution.

Next, we are concerned with the diffusion process of the 1,4-dioxane molecule in the oriented PBLG gel. We found the diffusion coefficients of 1,4-dioxane parallel and perpendicular to the α-helical PBLG axis to be 5.4×10^{-6} and 4.5×10^{-6}cm^2 s^{-1}, respectively. This difference is significant because the experimental error for the D value is estimated to be less than 5%. It is shown that the diffusion coefficient of the 1,4-dioxane molecule along the α-helical PBLG axis is significantly larger than that perpendicular to the α-helical PBLG axis. The diffusion process is thus anisotropic. This comes from the reason that because the degree of the

cross-linking is not very high, the obstacle for the translational displacement of 1,4-dioxane in the direction parallel to the α-helical axis is much larger than that in the direction perpendicular to the α-helical axis. From these experimental results in the oriented PBLG gel system, it can be said that the solvent dynamics is significantly influenced by the microstructure of the network polypeptide chains in the gel.

5. Conclusions

From the high-resolution solid-state ^{13}C NMR and pulsed-gradient spin−echo ^{1}H NMR experiments, the structure and diffusional behavior of both an oriented PBLG liquid crystalline solution and a PBLG gel were elucidated. It was found that the observed ^{13}C chemical shift, δ_{obs}, of the main-chain amide carbonyl carbons of PBLG becomes a reasonable parameter for determining the order parameter *S* of PBLG in the liquid crystalline solution, and further in the oriented PBLG gel, the diffusion behavior of the solvent in the oriented PBLG gel is anisotropic.

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