Diffusional Behavior of *n*-Alkanes in Polypeptide Gel System with Highly Oriented Chains As Studied by Pulsed Field Gradient Spin Echo ^{1}H NMR Method

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ABSTRACT: The diffusion coefficients (*D*) of *n*-alkanes, *n*-pentane and *n*-decane, and 1,4-dioxane as solvent in poly(γ-benzyl L-glutamate) (PBLG) gel with highly oriented α-helical chains were measured by pulsed field gradient spin echo ^{1}H NMR spectroscopy, of which the structure was determined by X-ray diffraction. From the experimental results, it was found that the diffusion coefficients of *n*-pentane and *n*-decane in the PBLG gel for the direction parallel and perpendicular to the magnetic field are $D_{\parallel} = 8.4 \times 10^{-6}$ cm^2/s and $D_{\perp} = 6.4 \times 10^{-6}$ cm^2/s, respectively, and $D_{\parallel} = 6.1 \times 10^{-6}$ cm^2/s and $D_{\perp} = 5.0 \times 10^{-6}$ cm^2/s, respectively, which correspond to the directions parallel and perpendicular to the α-helical main chain.

Introduction

In our previous papers,[1−3] we have studied the diffusional process of polypeptide gels by means of high-resolution solid-state ^{13}C NMR and pulsed field gradient spin echo (PFGSE) ^{1}H NMR methods. From these studies it has been elucidated that poly(γ-benzyl L-glutamate) (PBLG) gel with highly oriented chains was successfully prepared in an NMR magnet, and solvents in the gel diffuse anisotropically in restricted molecular motion.[3] Further, in anisotropic systems[4−7] such as lipids, synthetic liquid crystalline polymers, etc., different from polypeptide liquid crystal the diffusion process has been elucidated. Under such a background, we have been attracted to a research program how is the diffusional process of *n*-alkanes with the relatively extended conformation in the gel with anisotropic structure because of the fact that *n*-alkanes are basic molecules for understanding structure and dynamics of linear polymer chains.[8−10]

According to our previous work on the orientation of *n*-alkanes in PBLG liquid crystalline state by ^{1}H NMR,[11] in which PBLG chains are highly oriented, *n*-alkanes being guest molecules are highly oriented to the magnetic field of an NMR magnet with the relatively extended conformation along the α-helical PBLG chains. Also, as elucidated by NMR method, *n*-alkanes in the rotator phase take an extended conformation and are fastly rotating around the long axis,[12,13] and the diffusion coefficients for the direction in parallel and perpendicular to the long axis are different from each other.[14] Samulski et al.[15−17] and Abe et al.[18,19] have studied conformational behaviors of deuterated *n*-alkanes in PBLG liquid crystalline solution by conformational analysis combined the rotational isomeric state model and ^{2}H NMR spectral analysis. From these works, it has been shown that the orientational behaviors of guest alkanes in a liquid crystalline state have been clarified.

From the above-mentioned background, in this work we aim to elucidate the molecular packing of PBLG chains in the gel by means of wide-angle X-ray diffraction and to elucidate the diffusional process of guest *n*-alkane molecules, *n*-pentane and *n*-decane (n-C_5H_{12} and n-$C_{10}H_{22}$), in a PBLG gel with highly oriented chains by means of the PFGSE ^{1}H NMR method. These polypeptide gel samples were prepared by amide−ester exchange reaction in the strong magnetic field of an NMR magnet to be 10.5 T, to elucidate the diffusional process of guest *n*-alkanes.

Experimental Part

Materials and Gel Preparation. The poly(γ-benzyl L-glutamate) (PBLG) (M_w = 130 000) used in this work was supplied by Ajinomoto Co. Ltd. A 25 wt % PBLG/*n*-alkanes lyotropic liquid crystalline solution was prepared with 1,4-dioxane as 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 (10.5 T) used for NMR.[3] In the magnetic field PBLG chains are highly oriented. When the cross-linker was added to the PBLG solution in an NMR tube, the NMR tube was spun for several minutes in the NMR probe, to homogenize the cross-linker in the PBLG solution. Then, highly oriented PBLG gel obtained was swollen in 1,4-dioxane to remove the cross-linker.

Optical microscopic observation of the gel with highly oriented PBLG chains was made with an Olympus BH-2 polarizing microscope at room temperature. Figure 1 shows photographs of a 1,4-dioxane/*n*-decane (n-$C_{10}H_{22}$)/PBLG gel sample, which were taken by a polarzing microscope. From these photographs, it was recognized that the PBLG gel is highly oriented. The brightness regions are changed by changing the angle of an observation plate. This result gives us a clear evidence of the orientation structure of PBLG chains in 1,4-dioxane/*n*-decane (n-$C_{10}H_{22}$) /PBLG gel system.

Measurements. a. **NMR Measurements.** The self-diffusion coefficient measurements on 1,4-dioxane as solvent and *n*-pentane and *n*-decane as probe molecule in a PBLG gel were carried out by means of a JEOL GSX-270 NMR spectrometer operating at 270.1 MHz for ^{1}H with a homemade pulse gradient generator at 298 K. As the PFGSE pulse sequence, two field gradient pulses are added to the ($\pi/2$ pulse−τ−π pulse) sequence, in which the first field gradient pulse is added at the middle position between the $\pi/2$ and π pulses (that is, at the position of τ^* after the $\pi/2$ pulse), and the second at the position of τ^* after the π pulse.[20,21] A homemade pulse gradient generator is designed to generate the strong field gradient pulse (with a maximum field strength of about 22.8 T m^{-1}

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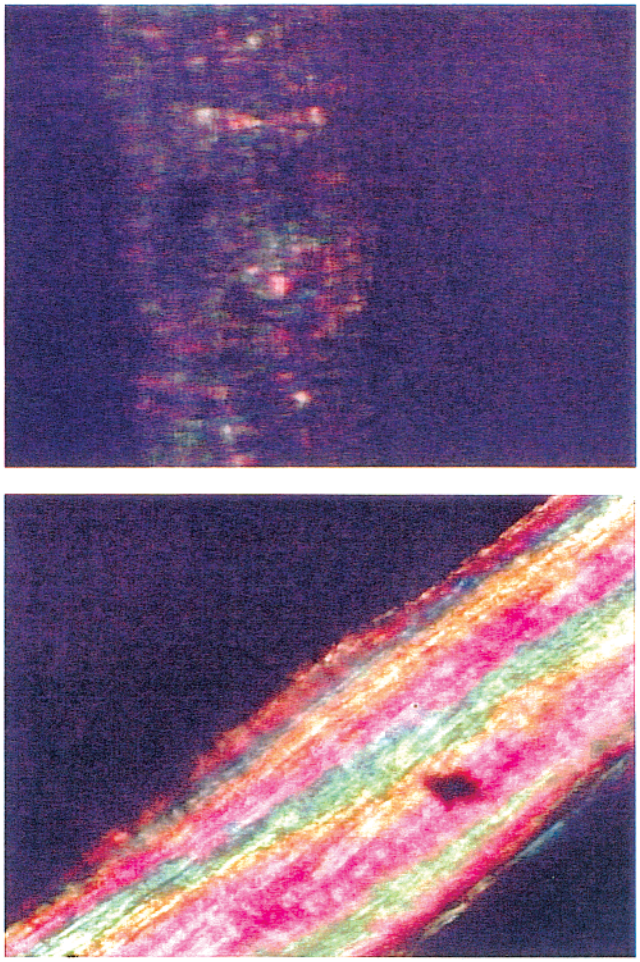

Figure 1. Cross-polarized optical microscope photographs of a PBLG/1,4-dioxane/*n*-decane (*n*-$C_{10}H_{22}$) gel with highly oriented PBLG chains which was made in an NMR magnet.

[2280 G/cm]) and to suppress the eddy currents induced. This has been successfully used in our previous works on diffusional behavior in polymer gel systems.[22−25] In this work, the field gradient strength of about 12.8 T m^{-1} [1280 G/cm] was used. The spectral width was 4.0 kHz, and there were 4096 data points. Such a pulse sequence has also been successfully used on gel systems by others[26−29] in our previous works.

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 ($\gamma = 2.675 \times 10^4$ 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 $\gamma^2 G^2 \delta^2(\Delta - \delta / 3)$ 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. The D value of 2.5×10^{-5} cm^2/s for water at 303 K was used as the calibration of the field gradient strength. The experimental error for the D value is estimated to be less than ca. 5%.

b. Wide-Angle X-ray Diffraction Measurements. Wide-angle X-ray diffraction patterns of PBLG gel samples and its dried samples were recorded with a flat-plate camera mounted to a Rigaku-Denki X-ray generator with Ni-filtered Cu Kα radiation. All of the experiments were made at room temperature (about 25 °C). In the dried sample experiments, after the gel sample was dried under vacuum for 3 days in order to remove solvent in the gel network, the obtained dried sample was in a glass capillary for X-ray diffraction measurements. On the other hand, in the polypeptide gel sample experiments the gel sample was sealed off in a glass capillary tube in order to avoid evaporation of solvent in the gel. Further, when we want to determine the intermolecular distance between the nearest-neighboring two PBLG chains in the gel as a function of network PBLG concentration, the gel sample was placed into a glass capillary tube without sealing off in order to obtain different network PBLG concentration by evaporation of solvent.

Results and Discussion

Orientation Analysis of Gel Network by Wide-Angle X-ray Diffraction. Wide-angle X-ray diffraction patterns were obtained for PBLG gels with highly oriented chains and their dried samples, to analyze the molecular packing of PBLG chains in the dried state and in the gel state. Parts a and b of Figure 2 show photographs of X-ray diffraction patterns for the dried sample and gel sample, respectively. In the dried sample the reflection (1) of network PBLG as shown in Figure 2a corresponds to the fifth layer line as observed in the solid PBLG sample (without cross-linking) with the α-helical (18_5 -helical) conformation that has the periodicity of 18 residues per 5 turns to be 5.09 Å. This means that in the PBLG gel with highly oriented chains the polypeptide chains take the α-helical conformation. This has also been justified by solid-state ^{13}C NMR results as reported previously.[3] From the reflection (2) of network PBLG as observed it is determined that the intermolecular distance between the nearest-neighboring two PBLG chains is 13.56 Å. On the other hand, in the gel sample, from the reflection (2) of network PBLG as shown in Figure 2b it is determined that the intermolecular distance between the nearest-neighboring two PBLG chains is 18.86 Å. Its intermolecular distance becomes larger than that in the dried state, as PBLG chains are swollen in solvent (1,4-dioxane). The molecular motion of the polypeptide chains in the gel becomes much higher than that in the dried state. Thus, the reflection that is corresponding to the fifth layer line could not be observed. Figure 3 shows a photograph of an X-ray diffraction pattern taken from the gel that was placed into a glass capillary without sealing off. The weight loss of the sample during the measurement is about 8.6 wt %. This indicates that the intermolecular distance between the nearest-neighboring two PBLG chains was changed from 18.86 to 17.03 Å. As solvent is evaporated from the gel, the intermolecular distance between the two nearest-neighboring PBLG chains becomes shorter. According to these experimental results, we can draw a structural diagram of the PBLG unit cell in the gel as shown in Figure 4.

Diffusional Behavior of Probe Molecules in the Liquid Crystalline Solution and Gel State. The plots of $\ln[A(\delta)/A(0)]$ against $\gamma^2 G^2 \delta^2(\Delta - \delta/3)$ for peaks of all of the diffusants in PFGSE ^1H NMR spectra become a straight line (not shown here). This shows that the diffusion of all of diffusants is a single component during the observation time. Then, the diffusion coefficients for all of the diffusants considered here were determined from the slope of the straight line as seen from eq 1. The determined diffusion coefficients (D) of 1,4-dioxane, *n*-pentane, and *n*-decane in the liquid crystalline state or in the gel with highly oriented PBLG chains are indicated in Table 1.

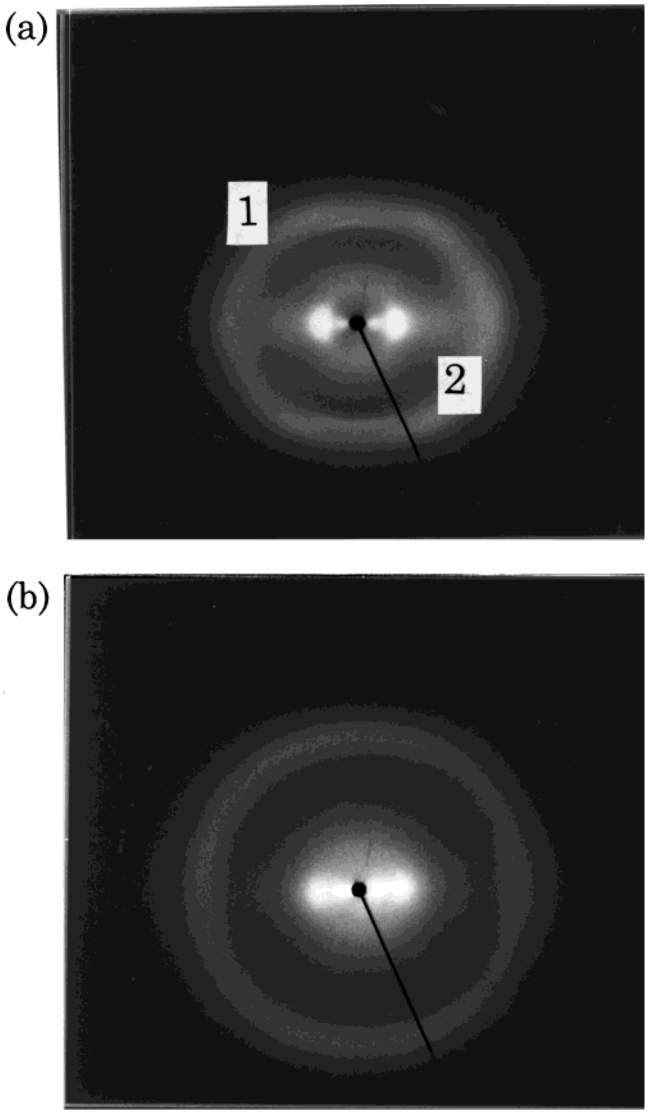

Figure 2. X-ray diffraction patterns of PBLG/1,4-dioxane/*n*-decane (n-$C_{10}H_{22}$) gel with highly oriented PBLG chains in the dried state (a) and in the gel state (b), which was prepared in an NMR magnet. The gel sample was sealed off into a glass capillary.

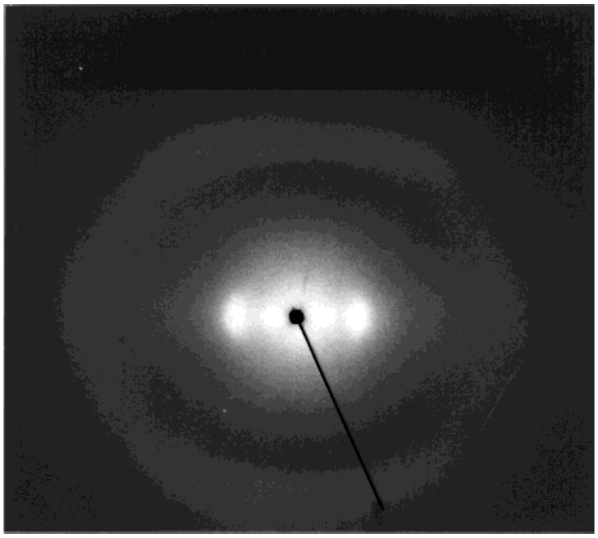

Figure 3. X-ray diffraction pattern of PBLG/1,4-dioxane/*n*-decane (n-$C_{10}H_{22}$) gel with highly oriented PBLG chains between which the nearest-neighboring intermolecular distance was changed by evaporating solvent. The gel sample was placed into a glass capillary without sealing off. The weight loss of the gel sample by evaporation of solvent was ca. 8.6 wt %.

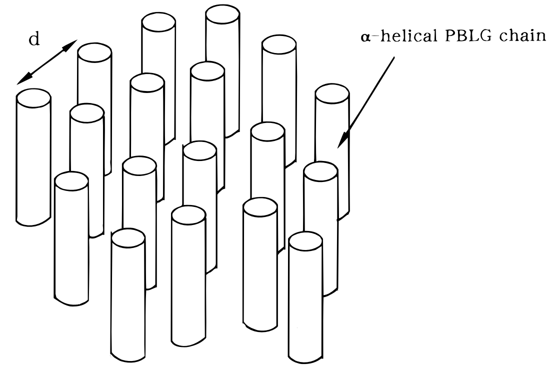

Figure 4. Schematic three-dimensional structure of highly oriented PBLG network in the gel state and in the dried state, where the intermolecular distances (d) between the nearest-neighboring two PBLG chains in the gel state and in the dried state are 18.86 and 13.56 Å, respectively.

Table 1. Determined Diffusion Coefficients ($\times 10^{-5}$ cm^2/s) of Dioxane and *n*-Alkanes in PBLG/1,4-Dioxane/*n*-Alkane Gel with Highly Oriented PBLG Chains, in the PBLG/1,4-Dioxane/*n*-Alkane Liquid Crystalline Solution and Pure Liquid State at 30 °C

	pure liq	liq cryst state	gel state		
molecule	D	D	$D_{\parallel}$	$D_{\perp}$	$(D_{\parallel} + 2D_{\perp})/3$
1,4-dioxane	1.06	0.58	0.54	0.45	0.48
n-pentane (n-C_5H_{12})	5.10	1.15	0.84	0.64	0.71
n-decane (n-$C_{10}H_{22}$)	0.96	0.69	0.61	0.50	0.54

The diffusion coefficients of 1,4-dioxane, *n*-pentane, and *n*-decane in pure liquid state as measured by the PFGSE ^{1}H NMR method are 1.06×10^{-5}, 5.1×10^{-5}, and 9.6×10^{-6} cm^2/s, respectively. The diffusion coefficient of *n*-pentane is much larger than that of 1,4-dioxane and of *n*-decane. The diffusion coefficients of 1,4-dioxane and *n*-decane are very close to each other. In the liquid crystalline state, the diffusion coefficients of 1,4-dioxane, *n*-pentane, and *n*-decane are 0.58×10^{-5}, 1.15×10^{-5}, and 0.69×10^{-5} cm^2/s, respectively. It is found that the diffusion coefficients of these molecules are largely reduced in the liquid crystalline solution as compared with those in pure liquid state.

In highly oriented gel system, it is expected from the previous works[11,14] that the diffusions of 1,4-dioxane, *n*-pentane, and *n*-decane are anisotropic. To make the diffusion coefficient experiments for justifying it, in the diffusion coefficient measurements, a highly oriented gel sample was placed in an NMR magnet as the PBLG helical chains in the corresponding gel orient in a direction parallel to the magnetic field and also perpendicular to the magnetic field. The diffusion coefficients corresponding to the former and the latter are indicated by $D_{\parallel}$ and $D_{\perp}$, respectively. The $D_{\parallel}$ values of 1,4-dioxane, *n*-pentane, and *n*-decane are 0.54×10^{-5}, 0.84×10^{-5}, and 0.61×10^{-5} cm^2/s, respectively. On the other hand, the $D_{\perp}$ values of 1,4-dioxane, *n*-pentane, and *n*-decane are 0.45×10^{-5}, 0.64×10^{-5}, and 0.50×10^{-5} cm^2/s, respectively. It is apparent that the diffusions of these molecules parallel to the PBLG helical chain in the network are significantly faster than that perpendicular to the PBLG helical chain in the network, that is $D_{\parallel} > D_{\perp}$, and so the diffusion is anisotropic as expected.

In previous work,[14b] the diffusion coefficients of n-$C_{24}H_{50}$ were measured in a lamellar single crystal at temperature of 49 °C in the rotator phase in the directions parallel and perpendicular to the long chain axis, which take an all-trans zigzag conformation. Then, the determined $D_{\parallel}$ and $D_{\perp}$ values were 1.64×10^{-6} and 2.70×10^{-6} cm^2/s, respectively, and so $D_{\parallel} < D_{\perp}$. This result is opposite to the above result in the highly oriented gel. This means that the translational migration of extended *n*-alkane chains in the direction perpendicular to the long axis is largely interrupted by networks, and it is different from the case of the diffusion in the rotator phase of pure alkane.

The $(D_{\parallel} + 2D_{\perp})/3$ value as calculated by using the $D_{\parallel}$ and $D_{\perp}$ values may be compared with the diffusion coefficients in pure liquid state and liquid crystalline state. The $(D_{\parallel} + 2D_{\perp})/3$ values of 1,4-dioxane, *n*-pentane, and *n*-decane are 0.48×10^{-5}, 0.71×10^{-5}, and 0.54×10^{-5} cm^2/s, respectively. The averaged diffusion coefficients become much smaller than those in pure liquid state and the liquid crystalline state. This means that in the gel system the translational motion of these diffusants was more strongly restrained by the interactions with the highly oriented PBLG chain network. Further, it can be said that an increase in the number of carbons of *n*-alkane leads to the reduction of the diffusion coefficients. As the probe molecules move through the gel network, the diffusion of probe molecules is strongly influenced by the size of probe molecules themselves.

Conclusions

It is concluded that the molecular packing of PBLG chains in PBLG gel samples and its dried samples with the highly oriented chains as prepared in an NMR magnet was successfully determined by using wide-angle X-ray diffraction, and the diffusion of solvent and *n*-alkanes in the corresponding gel as measured by the PFGSE ^{1}H NMR method is anisotropic.

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