Diffusion of Rodlike Polypeptides with Different Main-Chain Lengths in the Thermotropic Liquid Crystalline State As Studied by the Field-Gradient ^{1}H NMR Method

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ABSTRACT: The self-diffusion coefficients (D) of rodlike poly(n-alkyl L-glutamate)s having n-dodecyl side chains in the thermotropic liquid crystalline state were measured as a function of the main-chain length [molecular weight (M_w) of 7000, 30 000, and 130 000, which correspond to the main-chain lengths (L) of ca. 30, 200, and 890 Å, respectively)] within the temperature range from 50 to 80 °C by means of the pulse field-gradient spin-echo ^{1}H NMR method to elucidate the diffusional behavior of the polypeptides in the thermotropic liquid crystalline state. From the experimental results, it was found that at temperatures above the melting point of side-chain crystallites in poly(n-alkyl L-glutamate) the polypeptide forms the thermotropic liquid crystalline state, and then the isotropic diffusion coefficients (D_{iso}) of the rodlike polypeptides are decreased with an increase in the main-chain length. The diffusion process was analyzed by the Kirkwood theory of diffusion process for rodlike polymers. The diffusion coefficients of poly(L-glutamates) in the directions parallel ($D_{\|}$) and perpendicular ($D_{\perp}$) to the α-helical axis were determined, and the $D_{\|}$ value was found to be larger than the $D_{\perp}$ value.

Introduction

It is known that poly(γ-glutamate) with long n-alkyl side chains forms thermotropic liquid crystalline state by melting of the side-chain crystallites.[1] From solid-state high-resolution ^{13}C NMR experiments on poly(γ-n-alkyl L-glutamates),[2−6] it has been clarified that the main chain of polypeptides assumes a right-handed α-helical conformation and the n-alkyl side chains take an all-trans zigzag conformation in the crystallites at temperatures below the melting point and that at temperatures above the melting point of the side chain crystallites the side chains undergo fast trans−gauche exchange, and then the main chain undergoes fast molecular motion at a frequency of about 60 kHz.[5] Poly(n-dodecyl L-glutamate) (PDLG) forms a typical cholesteric liquid crystalline phase at temperatures above 50 °C. This shows that, by melting of the side-chain crystallites, the rate of reorientation of the side chains is transitionally increased. In previous work,[7] it was found that α-helical poly(γ-glutamate)s with n-octadecyl side chains in the thermotropic liquid crystalline state are diffusing as demonstrated by the field-gradient spin-echo (PFGSE) ^{1}H NMR method, and further the diffusion coefficients in directions parallel ($D_{\|}$) and perpendicular ($D_{\perp}$) to the α-helical chain axis were determined, and the diffusion was anisotropic. Further, it was demonstrated that the PFGSE NMR method[8−22] can be a useful tool for elucidating diffusion process of polymer gels,[23−27] polypeptides in the liquid crystalline state,[7] and n-alkanes in the "rotator phase", which is corresponding to the liquid crystalline state.[28]

From such a background, we aim to prepare highly oriented poly(γ-n-dodecyl L-glutamate) films as a function of the main-chain length and to measure the isotropic and anisotropic diffusion coefficients of the polypeptides as a function of temperature within the temperature range from 50 to 80 °C by means of the PFGSE ^{1}H NMR method to elucidate how the diffusion is affected by changes of the main-chain length. The diffusion behavior in the thermotropic liquid crystalline state is analyzed by the translational diffusion equation on the basis of Kirkwood theory[29] of the diffusion process for rodlike polymers derived by Doi and Edwards.[30]

Experiment

Materials. A series of poly(γ-n-dodecyl L-glutamate)s were synthesized by ester-exchange reactions between poly(γ-benzyl L-glutamate) (PBLG) (average molecular weight: $M_w = 7000$, 30 000, and 130 000) and n-dodecyl alcohol. The reaction was carried out in 1,2-dichloroethane with p-toluenesulfonic acid as a catalyst at 60 °C. The complete replacement of the phenyl groups of PBLG by n-alkyl groups was confirmed by their ^{1}H NMR spectra recorded in solution. The highly oriented PDLG films were prepared by casting the solutions in 1,2-dichloroethane at room temperature after placed in the magnetic field of an 11 T NMR magnet for 60 h. The orientation of the polypeptide chains in the film was confirmed by cross-polarized microscopy. The order parameter S of the obtained film is about 0.87 as determined by the ^{13}C chemical shift value of the amide carbonyl carbon[38] at room temperature.

Measurements. The self-diffusion coefficient measurements of poly(γ-n-alkyl L-glutamates) at the temperature range from 50 to 80 °C 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 using a standard PFGSE pulse sequence ($\pi/2$ pulse−τ−π pulse), which is the Hahn echo sequence,[8] and then two field gradient pulses are added between the $\pi/2$ and π pulses. In this work, a field-gradient strength of about 13.1 T m^{-1} (1310 G/cm) was used. The spectral width was 4.0 kHz, and the number of data points was 4096. The relationship between the echo signal intensity and pulse field-gradient parameters is given by[9]

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

where $A(\delta)$ and $A(0)$ are echo signal intensities at $t = 2\tau$ with and without the magnetic field gradient pulse, respectively. The field gradient pulse width 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}) gradient strength, D the self-diffusional coefficient,

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and Δ the field 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 $-D$. The τ, Δ, and δ values employed in these experiments were 10, 4, and 0.001−0.4 ms, respectively. The experimental error for the D value was estimated to be within 5%. The $D_{\parallel}$ and $D_{\perp}$ values were determined by rotation of the oriented polypeptide sample in an NMR probe which leads to change of the direction of field gradient.

Analysis of Diffusional Behavior of Rodlike Polypeptides. The main chain of poly(γ-glutamate) considered in this work takes the α-helical form like a long rod. At temperatures above the melting point of the side-chain crystallites the side chains take the liquidlike phase such as liquid n-alkane and are working as a solvent for the main chain. The rodlike polypeptide are diffusing as reported previously. The diffusion process of the polypeptide is assumed to follow the Kirkwood theory for the diffusion process of rodlike polymers.

The derivation of obtaining the translational diffusion coefficient of rodlike polymers was derived by Doi and Edwards[30] on the basis of Kirkwood theory;[29] the isotropic diffusion coefficient D_{iso} of a rodlike polymer chain is given by the following equation:

$$D_{\text{iso}} = (D_{\parallel} + 2D_{\perp})/3 = [\ln(L/b)/L]kT/3\pi\eta_{\text{s}} \tag{2}$$

in which

$$D_{\parallel} = [\ln(L/b)/L]kT/2\pi\eta_{\text{s}} \tag{3}$$

$$D_{\perp} = [\ln(L/b)/L]kT/4\pi\eta_{\text{s}} \tag{4}$$

where $D_{\parallel}$ and $D_{\perp}$ are the diffusion coefficients in parallel to and perpendicular to the rodlike polymer chain axis, respectively, L is the rodlike polymer length, b is the diameter of the rodlike polymer, η_{s} is the viscosity of the solvent corresponding to long n-alkyl side chains in the thermotropic liquid crystalline state, k is the Boltzmann constant, and T is the absolute temperature.

By using the standard bond lengths and bond angles determined by X-ray diffraction, we can straightforwardly estimate the rodlike main-chain length and the diameter of α-helical polypeptides. Then, the main-chain lengths of α-helical poly(γ-n-alkyl L-glutamate)s with average molecular weigths of 7000, 30 000, and 130 000 can be estimate to be L = ca. 30, 200, and 890 **Å**, respectively, and the diameter of the α-helical main chain including the ester group of side chain to b = ca. 10 Å. The diffusion process of these rodlike polypeptides is expected to follow eq 2. This equation shows that the plots of D_{iso} against $\ln(L/b)/L$ become a straight line.

Fundamental theories of diffusion for low-molecular-weight liquid crystals in the nematic phase have been studied by Franklin[31−33] based on a Oseen−Kirkwood hydrodynamic theory for isotropic liquids. Further theories of diffusion for low-molecular-weight liquid crystals have been developed. These theories explained partially the experimental data on $D_{\parallel}$ and $D_{\perp}$. Chu and Moroi[34] and Leadbetter[35] have obtained that the anisotropy ratio of the diffusion coefficients, $D_{\parallel}/D_{\perp}$, for low-molecular-weight liquid crystals are expressed by $[2\gamma(1 - S) + 2S + 1]/[\gamma(S + 2) + 1 - S]$, where $\gamma = \pi d/4l$ in which l is the length and d of the diameter of the rodlike molecules. This equation shows that if S becomes less than 1, $D_{\parallel}/D_{\perp}$ is reduced.

Results and Discussion

Diffusional Behavior of Poly(γ-*n*-dodecyl L-glutamate)s with Different Main-Chain Lengths. Spin-echo ^{1}H NMR spectra of unoriented poly(γ-n-dodecyl L-glutamate) [PDLG] films with different molecular weights of 7000, 30 000, and 130 000 in the thermotropic liquid crystalline state over the temperature range from 50 to 80 °C were measured as a function of field-gradient pulse length (δ) by using PFGSE ^{1}H NMR. In the spin-echo ^{1}H NMR spectra of PDLG with low molecular weight ($M_{\text{w}} = 7000$) an asymmetric sharp signal with a shoulder peak appears at about 1 ppm. The intense peak and the shoulder peak at about 0.9 ppm on right can be straightforwardly assigned to the inside methylene protons and the methyl protons, respectively, in the side chains.[7] The other minor signals at about 2 ppm may be assigned to the remaining methylene protons in the side chains. The peaks from the main-chain protons such as the α-CH and amide protons do not appear by great broadening because of the extremely slow molecular motion in the ^{1}H NMR time scale within the measurement temperature. However, in the spectra of the polypeptides with higher molecular weight only a single intense peak appears at about 1 ppm within the measurement temperature range.

Table 1. Determined Diffusion Coefficients of Poly(γ-dodecyl L-glutamate) at the Range of Temperature from 50 to 80 °C by the ^{1}H NMR Method

		temp (°C)						
mol wt	$D/\times 10^{-7}$ cm^2/s	50	55	60	65	70	75	80
7000	$D_{\parallel}$	17.6	18.3	19.1	19.7	20.4	21.2	21.8
	D_{iso}	16.7	17.3	18.1	18.9	19.9	20.6	21.3
	$D_{\perp}$	15.7	16.2	17.2	18.1	19.1	19.8	20.6
	$D_{\parallel}/D_{\perp}$	1.12	1.13	1.11	1.09	1.07	1.07	1.06
30 000	$D_{\parallel}$	10.4	11.1	11.9	12.8	13.6	14.3	15.4
	D_{iso}	9.21	9.96	10.7	11.7	12.7	13.5	14.5
	$D_{\perp}$	7.94	8.72	9.51	10.5	11.6	12.6	13.6
	$D_{\parallel}/D_{\perp}$	1.31	1.27	1.25	1.22	1.17	1.13	1.13
130 000	$D_{\parallel}$	6.33	7.33	8.44	9.31	10.6	11.4	12.1
	D_{iso}	5.67	6.44	7.56	8.43	9.63	10.5	11.2
	$D_{\perp}$	4.77	5.65	6.78	7.62	8.67	9.73	10.8
	$D_{\parallel}/D_{\perp}$	1.33	1.30	1.24	1.22	1.22	1.17	1.12

In the field-gradient spin-echo spectrum, the intensities of the peaks are reduced with an increase in field-gradient pulse duration (δ). This comes from the translational diffusion of the polypeptide chain within the observation time which is the time interval between two gradient pulses. This shows that the rodlike polypeptides are diffusing in the thermotropic liquid crystalline state. These results are the same as the experimental results in the previous work on the diffusional behavior of poly(γ-octadecyl L-glutamate) in the thermotropic liquid crystalline state. By using these decay signals, the diffusional coefficient D of PDLG can be determined from the slope of the plots of $\ln[A(\delta)/A(0)]$ against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ as listed in Table 1. As seen from this table, the diffusion coefficients of PDLG are decreased with an increase in the main-chain length. This shows that the diffusion coefficient of the longer rodlike polypeptides becomes much smaller. Such a diffusional behavior can be analyzed by using the translational diffusion equation of rodlike polymers derived by Doi and Edwards[30] on the basis of Kirkwood theory.[29]

The Main-Chain Length Dependence of the Diffusion Coefficients of the Rodlike Polypeptide Chain in the Thermotropic Liquid Crystalline State. As described above, the diffusion process of the rodlike polypeptides is expected to follow eq 2. This shows that the plots of D_{iso} against $\ln(L/b)/L$ become a straight line at any given temperature. Here, it is assumed that the viscosity of the solvent corresponding to long n-dodecyl side chains is independent of the main-chain length, which are undergoing rapid exchange between the trans and gauche conformations like liquid n-alkanes. In Figure 1 the plots of the isotropic diffusion coefficients of PDLG in the thermotropic liquid crystal-

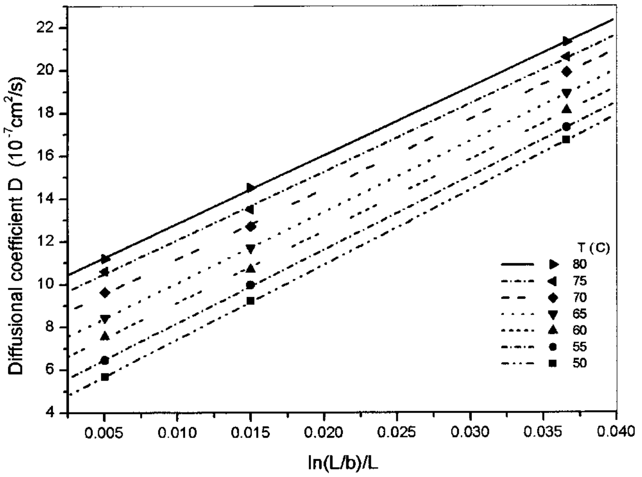

Figure 1. Plots of the isotropic diffusion coefficients of PDLGs in the thermotropic liquid crystalline state against ln(L/b)/L are shown at various temperatures.

line state against ln(L/b)/L are shown at various temperatures. It is found the plots become a straight line. This trend does not conflict with the theoretical prediction. Therefore, it can be said that the isotropic diffusion of rodlike PDLG chains follows approximately the translational diffusion equation of rodlike polymers derived by Doi and Edwards[30] on the basis of Kirkwood theory.[29] Further, it seen from Figure 1 that the slope of the plots of D_{iso} against ln(L/b)/L is increased with an increase in temperature. This agrees with the theoretical prediction as seen from eq 2 because the slope is a function of temperature.

Anisotropic Diffusion of Poly(γ-*n*-dodecyl L-glutamate) in the Thermotropic Liquid Crystalline State. The diffusion coefficients of the highly oriented polypeptide films for directions in parallel and perpendicular to the α-helical main chain were determined. In Table 1 the determined diffusion coefficients, $D_{\parallel}$ and $D_{\perp}$, of the polypeptides with different main-chain lengths and the ratio of $D_{\parallel}$ to $D_{\perp}$ are listed. From the table, it is seen that the $D_{\parallel}$ value is larger than the $D_{\perp}$ value. Their values are decreased with an increase in temperature. These agree with the previous results of poly(γ-*n*-octadecyl L-glutamate) in the thermotropic liquid crystalline state[7] and *n*-alkanes in the rotator phase.[28] In Figure 2 the plots of the isotropic diffusion coefficients of PDLG in the thermotropic liquid crystalline state against ln(L/b)/L are shown at various temperatures. It is found that the plots become a straight line. This trend does not conflict with the theoretical prediction. Therefore, it can be said that these experimental results can be qualitatively explained by the Kirkwood theory. As seen from Table 1, at 50 °C the ratio of $D_{\parallel}$ to $D_{\perp}$ is 1.33 and at 80 °C is 1.12. These values are close to those of low-molecular-weight liquid crystals[36] such as PAA (*p*-azoxyanisole) and DMBBA (*p*-methoxybenzylidene-*p*′-*n*-butylaniline) to be 1.33 and 1.44, respectively. At this stage, we do not have some clear reason why $D_{\parallel}/D_{\perp}$ is less than 2. As one of the possible reasons, it is considered that the effective medium viscosities in parallel and perpendicular direction to the rodlike polymer chain axis are different from each other, and then the former is somewhat smaller compared with the latter, so $D_{\parallel}/D_{\perp}$ is less than 2.

Activation Energy for Diffusion of Poly(γ-*n*-dodecyl L-glutamate) in the Thermotropic Liquid Crystalline State. To understand the diffusion process from a concept of the activation energy E for the diffusion of PDLG in the thermotropic liquid crystalline state, the following equation is used.

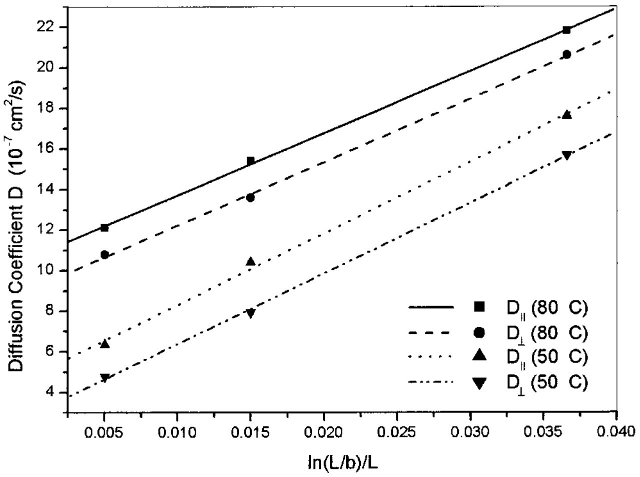

Figure 2. Plots of the diffusion coefficients $D_{\parallel}$ and $D_{\perp}$ of PDLGs in the thermotropic liquid crystalline state against ln(L/b)/L are shown at 50 and 80 °C, respectively.

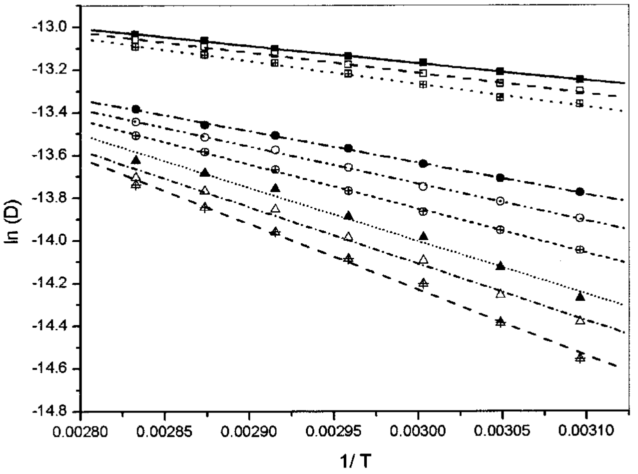

Figure 3. Arrhenius plots of ln D against 1/T for PDLGs with various molecular weights in the thermotropic liquid crystalline state: ■, ln($D_{\parallel}$); □, ln(D_{iso}); crossed ⊞, ln($D_{\perp}$) are for PDLG with molecular weight 7000; ●, ln($D_{\parallel}$); ○, ln(D_{iso}); ⊕, ln($D_{\perp}$) are for PDLG with molecular weight 30 000; ▲, ln($D_{\parallel}$); △, ln(D_{iso}); △, ln($D_{\perp}$) are for PDLG with molecular weight 130 000.

Table 2. Determined Activation Energy E of Polypeptides PG-12 at the Range of Temperature from 50 to 80 °C

		activation energy E (kJ/mol)		
mol wt	temp (°C)	$E_{\parallel}$	E_{iso}	$E_{\perp}$
7000	50−80	6.79	7.95	8.95
30 000	50−80	12.3	14.5	17.3
130 000	50−80	20.8	22.1	25.7

$$D = A \exp(-E/kT) \quad (5)$$

where A is the front factor, T the absolute temperature, and k the Boltzmann constant. The plots of ln D for polypeptide samples with three different chain lengths against T are shown in Figure 3. It is shown that these plots become a straight line. The activation energy E can be obtained from the plots of ln D against 1/T as listed in Table 2. From this table, the E values for the diffusion coefficients D_{iso}, $D_{\parallel}$, and $D_{\perp}$ are increased with an increase in the main-chain length. The E values for the diffusion coefficient $D_{\parallel}$ is larger than that for the

$D_{\perp}$. This agrees with the case of poly(γ-*n*-octadecyl L-glutamate) in the thermotropic liquid crystalline state.

Finally, we can conclude that at temperatures above the melting point of side-chain crystallites in poly(*n*-alkyl L-glutamate) the polypeptide forms the thermotropic liquid crystalline state, and then the diffusion coefficients of the rodlike polypeptides are decreased with an increase in the main-chain length and follow reasonably the Kirkwood theory of diffusion process for rodlike polymers. The diffusion coefficients of poly(L-glutamates) in the directions parallel ($D_{\parallel}$) and perpendicular ($D_{\perp}$) to the α-helical axis were determined, and the $D_{\parallel}$ values were found to be larger than the $D_{\perp}$ values.

References and Notes

(1) Watanabe, J.; Ono, H.; Uematsu, I.; Abe, A. *Macromolecules* **1985**, *18*, 2141.
(2) Yamanobe, T.; Tsukahara, M.; Komoto, T.; Watanabe, J.; Ando, I.; Uematsu, I.; Deguchi, K.; Fujito, T.; Imanari, M. *Macromolecules* **1988**, *21*, 48.
(3) Mohanty, B.; Komoto, T.; Watanabe, J.; Ando, I.; Shiibashi, T. *Macromolecules* **1989**, *22*, 4451.
(4) Tsukahara, M.; Yamanobe, T.; Komoto, T.; Watanabe, J.; Ando, I. *J. Mol. Struct.* **1987**, *159*, 345.
(5) Katoh, E.; Kurosu, H.; Ando, I. *J. Mol. Struct.* **1994**, *318*, 123.
(6) Mohanty, B.; Watanabe, J.; Ando, I.; Sato, K. *Macromolecules* **1990**, *23*, 4908.
(7) Yin, Y.; Zhao, C.; Kuroki, S.; Ando, I. *J. Chem. Phys.* **2000**, *113*, 7635.
(8) Hahn, E. L. *Phys. Rev.* **1950**, 580.
(9) Stejskal, E. O.; Tanner, E. J. *J. Chem. Phys.* **1965**, *42*, 288.
(10) Tanner, J. E.; Stejskal, E. O. *J. Chem. Phys.* **1968**, *49*, 1768.
(11) Nose, T. *Annu. Rep. NMR Spectrosc.* **1993**, *27*, 218.
(12) Price, W. S. *Annu. Rep. NMR Spectrosc.* **1996**, *32*, 53.
(13) Yasunaga, H.; Kobayashi, M.; Matsukawa, S.; Kurosu, H.; Ando, I. *Annu. Rep. NMR Spectrosc.* **1997**, *34*, 39.
(14) Matsukawa, S.; Yasunaga, H.; Zhao, C.; Kuroki, S.; Ando, I. *Prog. Polym. Sci.* **1999**, *24*, 995.
(15) Callaghan, P. T. *Principles of Nuclear Magnetic Resonance Microscopy*; Clarendon: Oxford, 1991.
(16) Kimmich, R. *NMR: Tomography, Diffusometry, Relaxometry*; Springer: Berlin, 1997.
(17) Bluemler, P., Bluemich, B., Botto, R., Fukushima, E., Eds.; *Spatially Resolved Magnetic Resonance*; Wiley-VCH Publisher: Weinheim, 1998.
(18) Callaghan, P. T.; Jolley, K. W.; Levierve, J.; King, R. B. K. *J. Colloid Interface Sci.* **1983**, *92*, 332.
(19) Ohtsuka, A.; Watanabe, T.; Suzuki, T. *Carbohydr. Polym.* **1994**, *25*, 95.
(20) Tanner, J. E. *J. Chem. Phys.* **1978**, *69*, 1748.
(21) von Meerwall, E.; Ferguson, R. D. *J. Chem. Phys.* **1981**, *74*, 6956.
(22) Callaghan, P. T.; Jolley, K. W.; Lelievre, J. *Biophys.* **1979**, *28*, 133.
(23) Callaghan, P. T.; Sonderman, O. *J. Phys. Chem.* **1983**, *87*, 1737.
(24) Zhao, C.; Kuroki, S.; Ando, I. *Macromolecules* **2000**, *33*, 4486.
(25) Matsukawa, S.; Ando, I. *Macromolecules* **1999**, *31*, 1865.
(26) Gao, Z.; Schlick, S.; Matsukawa, S.; Ando, I.; Rossi, G. *Macromolecules* **1999**, *32*, 3289.
(27) Matsukawa, S.; Yasunaga, H.; Zhao, C.; Kuroki, S.; Kurosu, H.; Ando, I. *Prog. Polym. Sci.* **1999**, *24*, 995.
(28) Yamakawa, H.; Matsukawa, S.; Kurosu, H.; Kurosu, S.; Ando, I. *J. Chem. Phys.* **1999**, *111*, 5129.
(29) Kirkwood, J. G. *J. Polym. Sci.* **1954**, *12*, 1.
(30) Doi, M.; Edwards, S. F. *The Theory of Polymer Dynamics*; Clarendon Press: Oxford, 1986; Chapter 8.
(31) Franklin, W. *Mol. Cryst. Liq. Cryst.* **1971**, *14*, 227.
(32) Franklin, W. *Phys. Lett.* **1974**, *48A*, 247.
(33) Franklin, W. *Phys. Rev.* **1975**, *A11*, 2156.
(34) Chu, K.-S.; Moroi, D. S. *J. Phys., Colloq.* **1975**, *36*, C1.
(35) Leadbetter, A. J.; Temme, F. P.; Heidemann, A.; Howells, W. S. *Chem. Phys. Lett.* **1975**, *34*, 363.
(36) Krueger, G. J. *Phys. Rep.* **1982**, *82*, 229.
(37) Halle, B.; Quist, P.; Furo, I. *Liq. Cryst.* **1993**, *14*, 227.
(38) Zhao, C.; Zhang, H.; Yamanobe, T.; Kuroki, S.; Ando, I. *Macromolecules* **1999**, *32*, 3389.

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