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Diffusional Behavior of Poly(*β*-benzyl L-aspartate) in the Rodlike, Random-Coil, and Intermediate Forms as Studied by High Field-Gradient ^{1}H NMR Spectroscopy

Sho Kanesaka, Kazuhiro Kamiguchi, Masahito Kanekiyo, Shigeki Kuroki, and Isao Ando*

Department of Chemistry and Materials Science, Tokyo Institute of Technology, International Research Center of Macromolecular Science, 2-12-1 Ookayama, Meguro-ku, Tokyo 152, Japan

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The diffusion coefficients of poly(*β*-benzyl L-aspartate)(PBLA) with the α-helix (rodlike) form in chloroform and the random-coil form in a mixture of chloroform and trifluoroacetic acid have been measured as a function of the PBLA concentration (C_{PBLA}) by using high field-gradient ^{1}H NMR. The PBLA concentration range for the former is from 0.11 to 9.20 wt % and for the latter it is from 0.20 to 25.2 wt %. From these experimental results, it is found that the diffusion coefficient of PBLA in the rodlike form is much smaller than that of PBLA in the random-coil form at the same PBLA concentration. This implies that the small diffusion coefficients of PBLA in the rodlike form as compared with those in the random-coil form come from the relatively large radius of gyration R_g. Further, it is found that the diffusional behavior of PBLA in the rodlike form is different in the three PBLA concentration regions, that is, region 1 ($C_{\mathrm{PBLA}} = 0.11{\sim}0.39$ wt %), region 2 ($C_{\mathrm{PBLA}} = 0.39{\sim}0.86$ wt %), and region 3 ($C_{\mathrm{PBLA}} = 0.86{\sim}9.20$ wt %). In the random-coil form, the diffusion is different in the two PBLA concentration regions, that is, region I ($C_{\mathrm{PBLA}} = 0.20{\sim}1.03$ wt %) and region II ($C_{\mathrm{PBLA}} = 1.03{\sim}25.2$ wt %). These diffusional behaviors in the rodlike form and in the random-coil form can be reasonably explained by Tinland−Maret−Rinaudo theory and de Gennes theory, respectively. Further, transitional change from the rodlike form to the random-coil form is discussed on the basis of the diffusional behavior.

Introduction

Poly(*β*-benzyl L-aspartate) (PBLA) considered in this work is one of the most popular synthetic polypeptides as well as poly(*β*-benzyl L-glutamate) (PBLG).[1] It is known that PBLA takes the left-handed α-helix form in a helical solvent such as chloroform. On the other hand, the addition of a coil solvent such as trifluoroacetic acid (TFA) to PBLA in a helical solvent leads to the breaking of hydrogen bonds in the α-helical main chain and then induces the transitional change from the α-helix to the random-coil form. The conformational change from the α-helix form to the random-coil form in solution has been elucidated by ^{1}H and ^{13}C nuclear magnetic resonance (NMR),[2−8] optical rotatory dispersion (ORD),[9,10] circular dichroism (CD),[11,12] and infrared (IR) spectroscopies.[13,14] Nevertheless, the diffusional behavior of PBLA in the rodlike and the random-coil forms has never been clarified, because it is not easy to measure the diffusion coefficients of a polypeptide in the α-helix and the random-coil form.

In previous papers, we have studied the diffusional behavior of rodlike poly(*γ*-glutamate) with long *n*-alkyl side chains in the thermotropic[15,16] and lyotropic liquid crystalline phases[17] by using the pulse field-gradient spin−echo (PFGSE) ^{1}H NMR method. It has been shown that the diffusion coefficients of the polypeptide chain in the rodlike form have been successfully determined in the direction parallel ($D_{\|}$) and perpendicular ($D_{\perp}$) to the long chain axis, and the $D_{\|}$ value is larger than the $D_{\perp}$ value. Further, it has been shown that the isotropic diffusion coefficients of rodlike polypeptides decrease with an increase in the main-chain length and that the diffusion process follows the Kirkwood theory[19] for rodlike polymers and the α-helical polypeptide is diffusing as a rod. The diffusional behavior of random-coil poly(diethylsiloxane) (PDES) in the isotropic phase, and the rodlike and random-coiled PDESs in the biphasic phase composed of the isotropic and the liquid crystalline regions has been studied by using PFGSE ^{1}H and ^{13}C NMR methods.[18] It has been shown that the PDES chains in the liquid crystalline region diffuse as a rod and the diffusion coefficient is larger than those in the isotropic region where the PDES chains diffuse as a random coil.

As above-mentioned, high field-gradient NMR spectroscopy has provided intimate information about the diffusion process and the whole structure of PBLA in the rodlike, random-coil, and intermediate forms through the observation of the diffusion coefficient of the polymer.[10−24] Therefore, in this work, we aim to measure the diffusion coefficients (D) of PBLA in solution by the PFGSE ^{1}H NMR method and to elucidate the diffusional behavior of PBLA in the rodlike and the random-coil forms as a function of PBLA concentration, and further the diffusional behavior of PBLA in the intermediate form between the rodlike and the random-coil forms as a function of TFA concentration. On the basis of these experimental results, the whole structure of PBLA in the intermediate form is characterized.

Experimental Section

Materials. PBLA (molecular weight $M_w = 59\,900$) was purchased from Sigma Chemical Co. and was used without further purification. Chloroform-*d* (99.7 atom % D with 1% tetramethylsilane) was purchased from Sigma Chemical Co. and was used as the helical solvent. Trifluoroacetic acid-*d* (TFA-*d*: 99.5 atom % D) was purchased from Aldrich Chemical Co. and was used as the coil solvent. For convenience, the weight fraction of TFA-*d* in a mixture of $CDCl_3$ and TFA-*d* is indicated by f_{TFA}. To prepare the α-helical PBLA solution

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* Corresponding author.

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with $f_{TFA} = 0$ wt %, a powdered PBLA sample was slowly dispersed in $CDCl_3$ at 50 °C for 1 h and then was completely dissolved. It was kept at about 10 °C overnight and was used for NMR measurements. To prepare the random-coiled PBLA solution with $f_{TFA} = 50$ wt %, powdered PBLA was dissolved in a mixture of $CDCl_3$ and TFA-*d*. It was kept at about 10 °C overnight and was used for NMR measurements.

Measurements. The NMR experiments were carried out by means of a Bruker Avance DSX-300 NMR spectrometer operating at 300.11 MHz for ^{1}H with a field-gradient system (with the maximum field-gradient strength: 1160 G/cm) at 20 °C. Pulsed field-gradient spin−echo sequence (PFGSE) developed by Stejskal and Tanner[25] based on the Hahn sequence[26] was employed for the diffusion coefficient measurements as reported previously.[15−24]

In PFGSE ^{1}H NMR measurements, typical experimental conditions are as follows. The echo signal intensity is measured by changing the gradient strength G from 0 to 1160 G/cm, and the gradient pulse interval Δ is 20 ms for PBLA in the rodlike and the random-coiled forms and is 6 ms for PBLA in the intermediate form. The gradient pulse width δ is 2 ms, and the recycle delay and the number of scans are 10 and 16 s, respectively.

If the diffusion component is able to be considered as a single diffusion component, the relationship between the echo signal intensity as a function of G ($A(G)$) and the pulse field-gradient parameters in the PFGSE method is given by[25,27−30]

$$\frac{A(G)}{A(0)} = \exp(-\gamma^2\delta^2G^2D(\Delta - \delta/3)) \tag{1}$$

where $A(0)$ is the signal intensity in the absence of gradient pulses, γ is the magnetogyric ratio, and D is the diffusion coefficient. The plots of $\ln[A(G)/A(0)]$ against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ give a straight line with a slope of $-D$. Therefore, the D value can be determined from this slope.

When the diffusion of probe molecules has two diffusion components within the measurement time, the echo signal intensity is given by a superposition of contributions from the individual diffusion components as expressed by

$$\frac{A(G)}{A(0)} = f_1 \exp[-\gamma^2G^2D_1\delta^2(\Delta - \delta/3)] + f_2 \exp[-\gamma^2G^2D_2\delta^2(\Delta - \delta/3)] \tag{2}$$

where D_i and f_i are the diffusion coefficient and the fraction of the *i*th diffusion component, respectively. Then, $f_1 + f_2 = 1$. The fractions for the fast and slow diffusion components can be determined from the intercept of the least-squares fitted straight line.

Analysis of Diffusional Behavior of PBLA in the Rodlike and Random-Coil Forms. As already mentioned, PBLA takes the left-handed α-helix form in chloroform and the random-coil form in TFA, respectively. The diffusion of an isolated rodlike PBLA chain is different from that of the random-coiled PBLA chain. It has been shown that the diffusion coefficient depends on the radius of gyration R_g of a polymer chain and that the larger the R_g value becomes, the smaller the diffusion coefficient is.[31] If the polymer is a straight rod, R_g is proportional to the contour length of the polymer. On the other hand, R_g of a polymer chain with the random-coil form is expressed as $R_g \sim M^{\nu}$ ($\nu = 0.5{\sim}0.6$).

The polymer-concentration dependence of the diffusion coefficient for a rodlike polymer chain in the unstiff limit (where the persistence length p is not infinite) has been reasonably explained by Tinland, Maret, and Rinaudo[32] as developed on the basis of de Gennes theory[33−35] for the random-coiled polymer as will be described below. In dilute solution the polymer chains are isolated from each other, the diffusion process follows the Kirkwood theory for the diffusion process of rodlike polymers as reported previously.[16] The isotropic diffusion coefficient D_{iso} of a rodlike polymer chain is given by[19,31]

$$D_{iso} = [\ln(L/b)/L]\mathrm{kT}/3\pi\eta_s \tag{3}$$

where L is the rodlike polymer length, b is its diameter, η_s is the viscosity of solvent, k is the Boltzmann constant, and T is the absolute temperature. In a semidilute solution, the polymer-concentration (c) dependence of the diffusion coefficient in a good solvent is given by

$$D \sim p^{-5}M^{-2}c^{-3} \tag{4}$$

where p is the persistence length. The intermolecular correlation length ξ decreases with an increase in c as shown by the relationship[32] $\xi = R_g(c^*/c)^{\nu}$, where c^* is the overlap concentration and ν takes any specified value (0.5−0.6), respectively.[33] In the new crossover concentration range, c_c, $\xi = 2p$ in the concentrated region as defined by Tinland, Maret, and Rinaudo,[31] the diffusion coefficient is independent of the polymer concentration.

The polymer-concentration dependence of the diffusion coefficient for the random-coiled polymer is theoretically predicted by de Gennes.[33,34] In dilute solution, each of the polymer chains is isolated thus intermolecular interactions may be ignored. Hence, the diffusion coefficient is independent of the polymer concentration in such a polymer concentration region, and the diffusion process for a rigid spherical polymer like the random-coiled polymer follows the Einstein−Stokes theory[31] as expressed by

$$D = \frac{kT}{6\pi\eta_s R_h} \tag{5}$$

where R_h is the hydrodynamic radius of the polymer corresponding to R_g. In semidilute solution, polymer chains are entangled with each other and thus the diffusion is extremely restricted. The restriction increases with an increase in polymer concentration, and thus the diffusion coefficient decreases. The polymer-concentration dependence of the diffusion coefficient in a good solvent is given by

$$D \sim M^{-2}c^{-1.75} \tag{6}$$

As mentioned above, the diffusion coefficient is predominantly governed by the exponential indication of c. The diffusion coefficients of PBLA over a wide range of polymer concentrations are analyzed by the Tinland, Maret and Rinaudo method[32] based on de Gennes theory[33−35] for random-coiled polymers.

Results and Discussion

Diffusional Behavior of PBLA in the Rodlike Form and the Random-Coil Form. Figure 1 shows typical PFGSE ^{1}H NMR spectra of PBLA in 0.7 wt % PBLA/$CDCl_3$ solution at $f_{TFA} = 0$ wt % (a) and in a mixture of TFA-*d* and $CDCl_3$ at $f_{TFA} = 50$ wt % (b) at 20 °C and at $\Delta = 20$ ms as a function of G, where G was changed from 0 to 800 G/cm for (a) and from 0 to 200 G/cm for (b), to determine the diffusion coefficient for PBLA in the rodlike and the random-coil forms. PBLA in $CDCl_3$ takes the rodlike form, and in a mixted solvent at $f_{TFA} = 50$ wt %, it takes the random-coil form. In spectrum in panel a, the phenyl and benzyl CH_2 peaks for PBLA appear at 7.1 and 4.8 ppm, respectively. The $^{\alpha}$CH, $^{\beta}CH_2$, and NH peaks do not appear because of large broadening due to slow molecular motion. The benzyl CH_2 peak at 4.8 ppm is observed as a negative peak that comes from the effect of geminal spin−spin coupling between the two CH_2 protons in the Hahn type spin−echo signal. In spectrum in panel b, the peaks of the phenyl, benzyl CH_2, and $^{\beta}CH_2$ protons for PBLA and the CF_3COOH proton, contained in deuterated TFA-*d* as solvent, appear at 7.1, 4.8, 2.8, and 11.1 ppm, respectively. As seen from the observed spectral pattern, the phenyl peak of PBLA is very sharp. This shows that the molecular motion of the phenyl rings is in fast

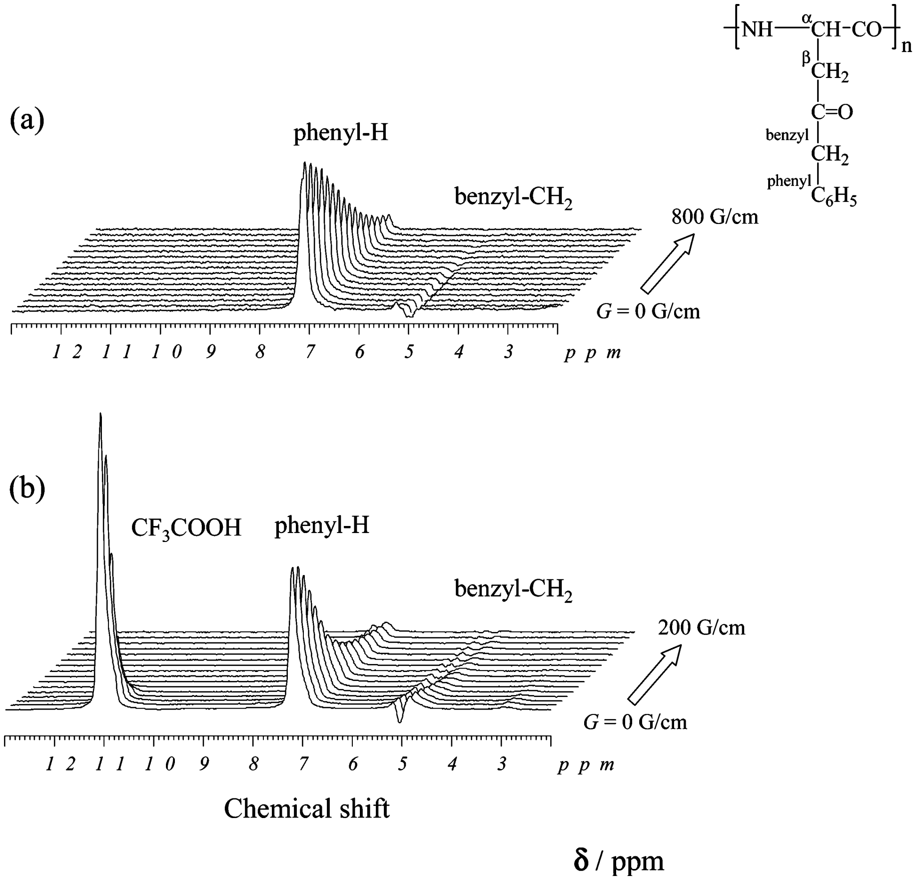

Figure 1. PFGSE ^{1}H NMR spectra of 0.7 wt % PBLA solution of $CDCl_3$ at f_{TFA}= 0 wt % (a) and 0.7 wt % PBLA solution of a mixture of $CDCl_3$ and TFA-*d* at f_{TFA}= 50 wt % (b) as a function of field-gradient strength *G* at 20 °C.

molecular motion as compared with that of PBLG.[36] Therefore, the phenyl peak can be used for the determination of the diffusion coefficient *D* of PBLA.

Further, it is seen from Figure 2 that the peak intensity decreases with an increase in field-gradient strength *G*, and the phenyl peak of PBLA in the random-coil form decreases faster than that in the rodlike form. This means that the random-coiled PBLA is diffusing much more rapidly than the rodlike PBLA. The diffusion coefficient *D* of PBLA in solution can be determined from the slope of the plots of $\ln[A(G)/A(0)]$ against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ by using the phenyl peak intensity. The experimental data lie on a straight line. This shows that the diffusion occurs with a single diffusion component within the observation time Δ in the polymer concentration range used. Polypeptide chains in a highly concentrated polymer solution interact with each other through the formation of hydrogen bonds, and often aggregate strongly. If PBLA chains aggregate, the corresponding diffusion component should be observed as a multidiffusion component. Thus, the PBLG chains at the polymer concentrations employed do not aggregate with each other.

The diffusion coefficients determined from the slope of a straight line as a single diffusion component for PBLA, in the rodlike and the random-coil forms, are plotted against the PBLA concentration *c* from 0.1 to 9.2 wt % for the rodlike form and from 0.2 to 25.2 wt % for the random-coil forms as shown in Figure 3. From the dependence of the diffusion coefficient of PBLA, in the rodlike form, on the PBLA concentration, it is apparent that there are three regions that have three different slopes. The difference between the slopes reflects that in the diffusion behavior between the diffusion processes of PBLA in these concentration regions. The three PBLA concentration regions are defined as region 1 at $c = 0.11\sim0.39$ wt %, region 2 at $c = 0.39\sim0.86$ wt %, and region 3 at $c = 0.86\sim9.20$ wt %. Each of the three slopes is determined by computer-fitting to be −0.80, −2.50, and −0.81 for regions 1, 2, and 3, respectively. In the same way, it has been found that there exist two regions for PBLA in the random-coil form to be region I at 0.20∼1.03 wt % and region II at 1.03∼25.2 wt %, which have slopes of −0.55 and −1.41, respectively. If the PBLA concentration is low enough, each of the PBLA chains isolates, and they do not interact with each other. Therefore, the diffusion coefficient should be independent of the PBLA concentration in such a region. However, the diffusion coefficients are weakly dependent on the PBLA concentration in the region 1 (the rodlike form) and region I (the random-coil form). The small concentration dependence in these regions comes from the fact that the center-of-mass of a PBLA chain within the observation time Δ is displaced as observed in the PFGSE NMR method. For example, the diffusion coefficient of a PBLA chain in the random-coiled form is $D = 2.67 \times 10^{-6}$ cm^2/s at 0.20 wt %. Therefore, the displacement of a PBLA chain may thus be estimated to be 3.27 μm by using the relation $(2D\Delta)^{1/2}$ within the observation time $\Delta = 20$ ms. This means that PBLA chains collide with each other. Therefore, the diffusion in these regions shows such a small polymer-concentration dependence. The slopes of −2.50 in region 2 (the rodlike form) and −1.41 in region II (the random-coil form) are close to −3 and −1.75, respectively, as predicted theoretically in the semidilute solution region. The slopes in region 1 and region 2 (the rodlike form) can be explained by the same argument as in the case of regions I and II. However, region 3 is completely different from the case in the random-coiled form. It can be said that the effect of entanglements between polymer chains on the diffusion becomes

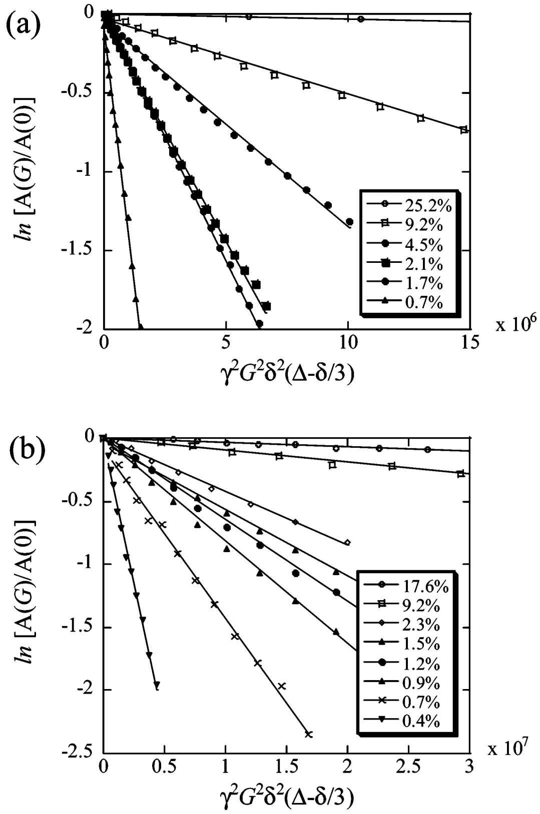

Figure 2. Plots of ln[$A(G)/A(0)$] against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ for determining of the diffusion coefficient D of PBLA in 0.7 wt % PBLA solution of $CDCl_3$ at f_{TFA}= 0 wt % (a) and in a mixture of $CDCl_3$ and TFA-d at f_{TFA}= 50 wt % (b) as a function of the PBLA concentration as obtained from PFGSE ^{1}H NMR experiments at 20 °C.

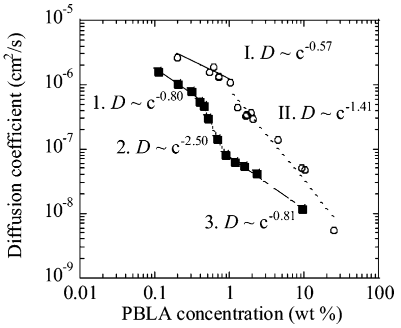

Figure 3. log−log plots of the diffusion coefficients of PBLA in $CDCl_3$ at f_{TFA}= 0 wt % (○) and PBLA in a mixture of $CDCl_3$ and TFA-d at f_{TFA} = 50 wt % (■) against the PBLA concentration at 20 °C as obtained from PFGSE ^{1}H NMR experiments.

stronger with an increase in polymer concentration, which leads to the prevention of the diffusion. However, the slope of polymer-concentration dependence in region 3 is smaller than that in region 2. This means that the effect of entanglements on diffusion does not increase more efficiently in region 3 as compared with region 2.

Rodlike polymer chains which have $p = \infty$ correspond to the stiff limit form and thus have an ordered structure in the concentrated solution region, that is, the liquid crystalline phase. Then, there are no entanglements in the diffusion process. However, the PBLA chains considered here do not form a liquid crystalline phase. Nevertheless, it is considered that the chains form a partially ordered structure in region 3 and do not entangle even if the PBLA concentration is increased. Therefore, the slope becomes smaller than that in region 2.

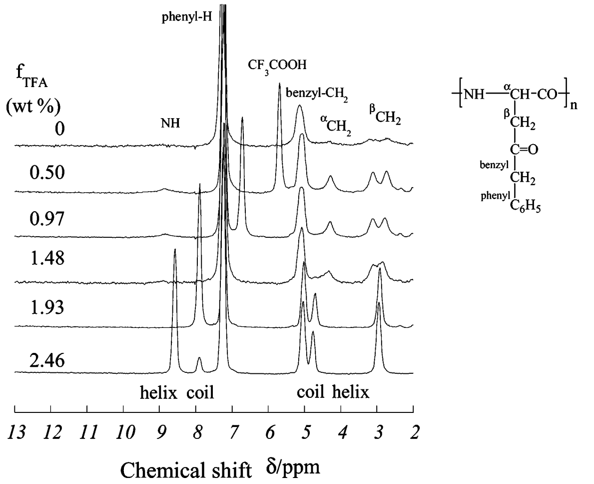

Figure 4. ^{1}H NMR spectra of 0.7 wt % PBLA solution of a mixture of $CDCl_3$ and TFA-d as a function of f_{TFA} at 20 °C.

The diffusion coefficient of PBLA in the rodlike form is smaller than that in the random-coil form at the same concentration. Thus, it can be said that PBLA in the rodlike form is in a semidilute region at lower concentrations than that in the random-coil form. This arises from the difference in the radius of gyration between the rodlike form and the random-coil form. The former is much larger than the latter. Moreover, specific behavior for PBLA in the rodlike form is observed in region 3, but on the other hand, there is no change in the slope for PBLA in the random-coil form at the same concentration range. As reported previously, the difference in the diffusion coefficient between the PDES chains in the rodlike form and the random-coil form comes from whether there exists entanglements or not.[18] However, in PBLA solution the radius of gyration plays an important role for the diffusional behavior.

Diffusional Behavior of PBLA in an Intermediate Form between the Rodlike and the Random-Coil Forms. The PBLA chains change from the rodlike to the random-coil form by the addition of a coil solvent into a PBLA solution with a helical solvent. To obtain diffusional information about the transition process from the rodlike to the random-coil form, ^{1}H NMR spectra of PBLA in a mixture of $CDCl_3$ and TFA-d were observed as a function of f_{TFA}, as shown in Figure 4 where the PBLA concentration is fixed to be 0.7 wt %. As shown in Figure 4 the positions of the peaks in the observed spectra are closely associated with the conformational transition. The position of TFA peak moves from 5.7 to 8.6 ppm with an increase in f_{TFA}. This arises from interactions between the PBLA chains and the TFA molecules. Actually, it is well-known that the positions of the amide NH and $^{\alpha}$CH peaks are strongly influenced by the transitional change from the α-helix form to the random-coil form. Therefore, the amide NH and $^{\alpha}$CH peaks in PBLA move from 8.8 to 7.9 ppm and from 4.2 to 4.8 ppm, respectively, at f_{TFA} = ca. 1.5 wt %. This behavior reflects the formation of the coil part, of which the amount is enough to detect by using ^{1}H NMR spectrum.

The PFGSE ^{1}H NMR experiments have been carried out as a function of f_{TFA} from 0 to 50 wt % at a diffusion time Δ = 6 ms by varying G. Within the TFA concentration range, the viscosity of solvent is assumed to be almost constant. Then, the PBLA concentration is fixed at a low PBLA concentration of 0.7 wt % to avoid complicated intermolecular interactions occurring between PBLA and the solvent. The plots of ln[$A(G)/A(0)$] against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ by using the PFGSE signals of PBLA are shown in Figure 5, and the diffusion coefficient D

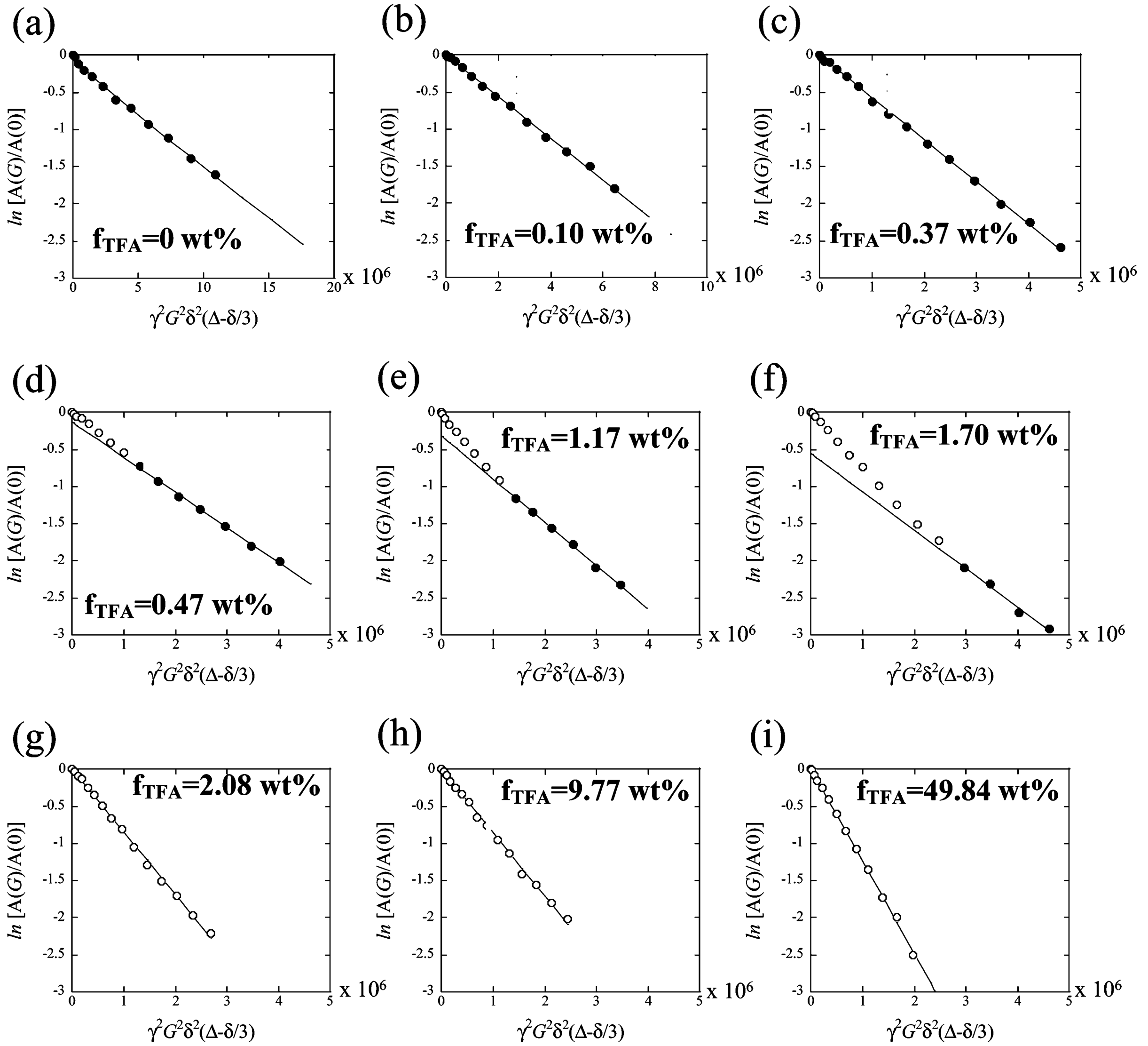

Figure 5. Plots of ln[$A(G)/A(0)$] against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ for determining of the diffusion coefficient D of PBLA in 0.7 wt % PBLA solution of a mixture of $CDCl_3$ and TFA-d as a function of f_{TFA} (a−i) as obtained from PFGSE ^{1}H NMR experiments at 20 °C.

and fraction f for the fast and slow diffusion components, as determined from the slope of the corresponding straight lines and their intercept, respectively, are plotted against f_{TFA} in Figure 6.

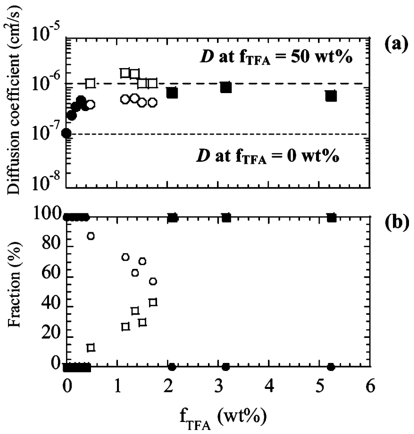

Figure 6. Plots of the diffusion coefficients of PBLA in 0.7 wt % PBLA of a mixture of $CDCl_3$ and TFA-d for the fast and slow diffusion components and their fractions against f_{TFA} as obtained from PFGSE ^{1}H NMR experiments at 20 °C. ●, the slow diffusion components observed as single diffusion component; ■, the fast diffusion components observed as single diffusion component; ○, the slow diffusion components observed as two diffusion components; and □, the fast diffusion components observed as two diffusion coefficients.

The dotted and broken lines in Figure 6a show the diffusion coefficient of PBLA at $f_{TFA} = 0$ wt % corresponding to the rodlike form and at $f_{TFA} = 50$ wt % corresponding to the random-coil form. As shown in Figure 5a−c, the plots of ln[$A(G)/A(0)$] for $f_{TFA} = 0\sim0.37$ wt % PBLG solutions against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ show a straight line. In this concentration range, the diffusion coefficient becomes faster, from 1.3×10^{-7} cm^2/s at $f_{TFA} = 0$ wt % to 5.7×10^{-7} cm^2/s at $f_{TFA} = 0.37$ wt %, and the chemical shift of the NH and $^{\alpha}CH_2$ protons do not change. From such behavior, it is expected that the small amount of TFA added to the helical solvent breaks hydrogen bonds in the PBLA main chain and then leads to the formation of a coil part. A PBLA chain can bend significantly by the formation of a coil part, even if it is only a small amount of coil, then the R_g of the PBLA chain becomes smaller. As a result, the diffusion coefficient becomes larger. Such behavior is supported by the broken-rod theory[36] and Zimm-Bragg-Nagai theory.[38] In $f_{TFA} = 0.47\sim1.70$ wt %, the plots of ln[$A(G)/A(0)$] against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ consist of two straight lines with different slopes as seen from Figure 5d−f. This shows that the diffusion contains two kinds of components, such as the fast and slow diffusion components. The diffusion coefficients for the fast and slow components are almost independent of f_{TFA}, and the fractions of the fast and slow diffusion components increase and decrease with an increase in f_{TFA}, respectively. The diffusion coefficients of the fast and slow components are very close to those in the random-coil form and the broken-rod form including a small amount of a coil part, respectively. This means that the broken-rod chain cooperatively changes to the random-coiled chain as f_{TFA} increases in this region. In $f_{\mathrm{TFA}} = 2.08 \sim 49.84$ wt %, the plots of $\ln[A(G)/A(0)]$ against $\gamma^2 G^2 \delta^2 (\Delta - \delta/3)$ remains a straight line as shown in Figure 5g−i. The determined diffusion coefficient from the slope is very close to that of PBLA in the random-coil form. Therefore, it can be said that the PBLA chains change to the random-coil form in this region.

It has been difficult to observe from the ^{1}H NMR spectrum information on the formation of a small amount of a coil part in the main chain. However, the change in R_g induced by the formation of a coil part can be observed as a change in the diffusion coefficient as determined by PFGSE ^{1}H NMR. It can be said that PFGSE ^{1}H NMR can give useful information about the whole structure of a polymer chain in the intermediate form.

Finally, we must describe briefly the PFGSE ^{1}H NMR measurements of PBLG with the helix form in solution to obtain information on the diffusional behavior, because the structure and dynamics of PBLG have been employed together with PBLA in the field of synthetic polypeptides. In this experiment, we could not observe significant PFGSE ^{1}H NMR spectra of PBLG with the helix form in dilute and concentrated solutions, because of extremely broad signals of the main chain and side chains, which come from much slower motion of the main chain and side chains compared with PBLA. For this, we cannot compare the diffusional behavior of PBLA obtained here with that of PBLG. This will be future work.

Conclusions

We can conclude as follows; the diffusions of PBLA chains in the α-helix and the random-coil forms are strongly influenced by the radius of gyration, and are reasonably followed by the Tinland-Maret-Rinaudo and de Gennes theories, respectively. On the other hand, the whole structure of PBLA chains in the intermediate form, between the rodlike and the random-coil forms, is clarified through the observation of their diffusional behavior. Further, it can be said that the PFGSE NMR method can be a useful means not only for studying the diffusional behavior of polymers, but also for characterizing the whole structure of polymers.

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