

Diffusional Behavior of Poly(γ -benzyl L-glutamate) in Concentrated Solution As Studied by the Field-Gradient ^1H NMR Methods

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The diffusion coefficient (D) of poly(γ -benzyl L-glutamate)(PBLG) in the isotropic, nematic and columnar phases in concentrated solution is successfully determined by the field-gradient ^1H NMR methods. The D values of PBLG molecules in the isotropic phase in 25% PBLG/1,4-dioxane solution are a little bit larger than the identical calculated D values of a PBLG α_R -helical chain by the Kirkwood theory, which means PBLG chains move independently in the isotropic phase in 25% PBLG/1,4-dioxane solution. Whereas the D values of PBLG molecules in other phases in 25% PBLG/1,4-dioxane solution and that in all phases in 35% 1,4-dioxane solution are smaller than the identical calculated D values of a PBLG α_R -helical chain. It means several PBLG chains move cooperatively in these phases. In the nematic phase, the PBLG molecules diffuse cooperatively as one *long rod* which containing the several PBLG chains. In the columnar phase, several PBLG chains diffuse also cooperatively keeping with two-dimensional order.

KEY WORDS: Poly(β -benzyl L-glutamate) / α_R -Helix / Lyotropic Liquid Crystal / Diffusion Coefficient / Field-gradient NMR /

It is well known that poly(glutamate)s with long n-alkyl side chains form thermotropic liquid crystalline state by melting of the side-chain crystallites and also poly(glutamate)s such as poly(γ -benzyl L-glutamate)(PBLG), poly(γ -n-alkyl L-glutamate), etc. in solvent form the isotropic, biphasic and liquid crystalline phases such as the cholesteric and columnar phases¹ depending on the polypeptide concentration. In our previous works, we have successfully measured the diffusion coefficients of rod-like polypeptides such as α -helical poly(γ -glutamate)s having long n-alkyl side chains as a function of the α -helical chain length in the thermotropic liquid crystalline state^{2,3} and of α -helical poly(γ -n-octadecyl L-glutamate) and chloroform as solvent in the isotropic, biphasic and liquid crystalline phases⁴ by high field-gradient ^1H NMR method. Most recently, the diffusional behavior of Poly(β -benzyl L-aspartate) (PBLA) in the rod-like, random-coiled and intermediate forms is reported⁵ and it is shown that the diffusional behaviors in the rod-like form and in the random-coiled form can be reasonably explained by Tinland-Maret-Rinaudo theory and de Gennes theory, respectively. Though there is no diffusion study for PBLG in the lyotropic liquid crystalline state by the field-gradient ^1H NMR method, because the ^1H signals of PBLG in the lyotropic liquid crystalline state are very broaden by the very short ^1H T_2 ⁶ and it is difficult to observe ^1H spectrum. Especially for the case of DP (degree of polymerization) over 1000, the broadening becomes so extreme that no spectrum at all is evident.

The diffusion for PBLG in a dilute solution was studied by dynamic light scattering (DLS),^{7,8} fluorescence photobleaching recovery(FPR)⁹ and rheo-optical technique.¹⁰ But there is no study for a diffusional behavior of PBLG in the liquid crystalline phases in a concentrated solution.

Lyotropic liquid crystalline phases have been extensively studied in biological molecules, because the liquid crystalline field adds in self-organization of rod-like biopolymers that may be important for functionality in biological systems. New liquid crystalline phases such as the columnar and smectic phases have been reported in lyotropic liquid crystals of synthetic polypeptides,^{1,11–14} DNA^{15,16} and tobacco mosaic virus.¹⁷ More recently, Yen *et al.*¹⁸ found a clear transition between the cholesteric and columnar phases in addition to the well-known isotropic to cholesteric phase transition in the poly(γ -phenethyl L-glutamate)/m-crezol system by the visual and microscopic observations and the X-ray measurement, and constructed the whole phase diagram in the concentration range of 0 to 100 vol% and the temperature range of 0 to 70 °C. Similar phase diagrams are presumed for other poly(γ -glutamate)s such as PBLG lyotropic systems.

In this work, the self-diffusion coefficients(D) of PBLG molecules in the isotropic, nematic, and columnar phases are determined by the field-gradient ^1H NMR methods at various temperatures and the diffusional behavior of rod-like PBLG molecules in the several phases in concentrated solution is successfully elucidated.

EXPERIMENTAL

Materials

PBLG (DP (degree of polymerization) = 463, M_w (Molecular weight) = 101,400) was purchased from Sigma Chemical Co. As the solvent, 1,4-dioxane was used as supplied from Kanto Chemical co. Ltd. The PBLG solutions were prepared by placing PBLG and 1,4-dioxane in an NMR tube with a diameter of 10 mm. The PBLG concentrations were 35%

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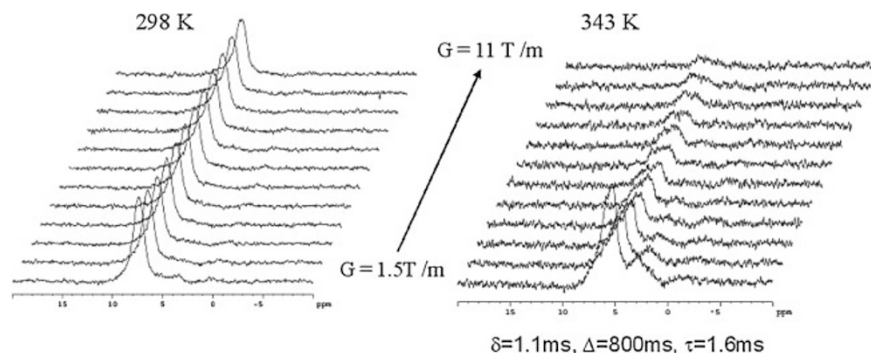


Figure 1. ^1H PGStE NMR spectra of PBLG in 35% PBLG/1,4-dioxane solution at 298 and 333 K as a function of the field gradient strength G .

(0.35 g of PBLG with 0.65 g of 1,4-dioxane) and 25% (0.25 g of PBLG with 0.75 g of 1,4-dioxane). In PBLG/1,4-dioxane solutions, a PBLG molecule takes an α_R -helical form.

NMR Measurements

The NMR experiments were carried out by means of a Bruker Avance DSX 300 NMR spectrometer operating at 300.11 MHz for ^1H with field-gradient generator system (with the maximum field-gradient strength: 11.60 T/m). Pulsed field-gradient stimulated-echo sequence (PGStE) ($\pi/2$ pulse $-\tau_1$ - $\pi/2$ pulse $-\tau_2$ - $\pi/2$ pulse $-\tau_1$ -echo) was employed for diffusion coefficient measurements as reported previously.^{19–23}

In PGStE ^1H NMR measurements, a typical experiment condition is as follows. The echo signal intensity is measured by changing the gradient strength G from 1.5 to 11 T/m, and the gradient pulse interval Δ is 800 ms, and the two gradient pulse widths δ are 1.1 ms in the two τ_1 periods which are 1.6 ms and the recycle delay is 2 or 4 s.

The PBLG/1,4-dioxane solutions are placed in NMR magnet for 1 night before the NMR measurements, so the molecular axis of PBLG chains align with an external magnetic field B_0 and the cholesteric phase transfers to the nematic phase.

If the diffusion component is able to be suppose as the 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 PGStE method is given by

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

where $A(0)$ is the signal intensity in the absence of gradient pulses, γ the magnetogyric ratio and D 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 the 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

$$\frac{A(G)}{A(0)} = f_1 \exp\left(-\gamma^2\delta^2G^2D_1\left(\Delta - \frac{\delta}{3}\right)\right)$$

$$+ f_2 \exp\left(-\gamma^2\delta^2G^2D_2\left(\Delta - \frac{\delta}{3}\right)\right) \quad (2)$$

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. But the fractions of the corresponding diffusion coefficient components f_i are not correct existent ratios, because the initial signal intensities decrease by ^1H T_2 in τ_1 period and decrease by T_1 in τ_2 period of PGStE ($\pi/2$ - τ_1 - $\pi/2$ - τ_2 - $\pi/2$ pulse(- τ_1 -echo)) method.

RESULTS AND DISCUSSION

Diffusional Behavior of PBLG in 35% PBLG/1,4-dioxane Solution at Various Temperature

Figure 1 shows a typical ^1H PGStE NMR spectra of 35% PBLG/1,4-dioxane solution at 298 and 333 K as a function of the field gradient strength G . The observed signal appeared at 7 ppm is assigned to the phenyl protons of PBLG. The ^1H signals assigned to the protons of 1,4-dioxane disappear in this experimental condition ($G > 1.5$ T/m), because of the larger self-diffusion coefficient (D) of 1,4-dioxane compared with PBLG. So only the ^1H signals assigned to the protons of PBLG can be observed. To obtain the self-diffusion coefficients (D) of PBLG in 35% PBLG/1,4-dioxane solution at wide temperature range, ^1H PGStE NMR measurements are performed from 298 to 333 K. Figure 2 shows the plots of the echo attenuation of PBLG in 35% 1,4-dioxane solution from 298 K to 333 K. From the decay of signal attenuation, it seems that the experimental data do not lie on a straight line but on a curve decomposed into two components expressed by equation 2 at each temperature. This means that two kinds of diffusion component coexist at each temperature. The determined D values are listed in Table I together with the fractions of the corresponding diffusion coefficient components obtained by the decay of signal attenuation shown in Figure 2. Figure 3 shows the plots of the self-diffusion coefficients of PBLG molecules in 35% PBLG/1,4-dioxane solution against temperature. It is found that the slower diffusion coefficient gradually increases with increasing temperature, but that a clear

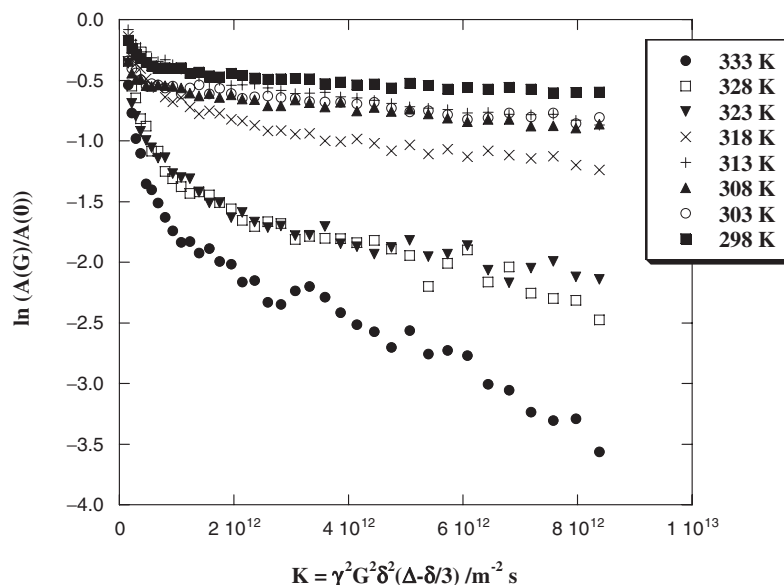


Figure 2. Plots of echo attenuation of PBLG in 35% 1,4-dioxane solution at 298 K to 333 K.

Table I. Determined diffusion coefficients (D)^a PBLG in 35% PBLG/1,4-dioxane solution at various temperature with the fraction (f)^b of the corresponding diffusion coefficient components

Temperature/K	Diffusion coefficients/m ² /s		
	$D_{\text{isotropic}}(f_{\text{isotropic}})$	$D_{\text{nematic}}(f_{\text{nematic}})$	$D_{\text{columnar}}(f_{\text{columnar}})$
298	3.98×10^{-12} (0.33)		2.43×10^{-14} (0.67)
303	6.36×10^{-12} (0.38)		3.85×10^{-14} (0.62)
308	7.71×10^{-12} (0.42)		4.46×10^{-14} (0.58)
313		1.84×10^{-12} (0.34)	4.98×10^{-14} (0.66)
318		2.65×10^{-12} (0.51)	6.48×10^{-14} (0.49)
323		3.67×10^{-12} (0.58)	6.78×10^{-14} (0.42)
328		3.48×10^{-12} (0.73)	1.27×10^{-13} (0.27)
333		4.83×10^{-12} (0.80)	2.18×10^{-13} (0.20)

^aThe experimental errors for the diffusion coefficient measurements are ca. 10%. ^bThe experimental errors for the fraction of the components are ca. 1%.

transition of the faster diffusion coefficient exists between 308 K and 313 K.

From the phase diagram of Poly(γ -phenethyl L-gulutamate)/m-crezol system,¹⁸ it assumes that there exist the isotropic and columnar phases below 308 K and there exist the nematic (in a magnetic field, the cholesteric phase transfers to the nematic phase) and columnar phases above 313 K in 35% PBLG/1,4-dioxane system. Accordingly the component which has a slower diffusion coefficient D is assigned to the columnar phase at all temperature range. The component which has a faster diffusion coefficient D is assigned to the isotropic phase below 308 K and to the nematic phase above 313 K. We show these assignments in Table I and Figure 3. The $D_{\text{isotropic}}$ are 3.98 to 7.71×10^{-12} m²/s, the D_{nematic} are 1.84 to 4.83×10^{-12} m²/s and the D_{columnar} are 2.43×10^{-14} to 2.18×10^{-13} m²/s, respectively. The fractions of the corresponding diffusion coefficient components in Table I are not correct existent ratios, because the initial signal intensities (f_i of equation (2))

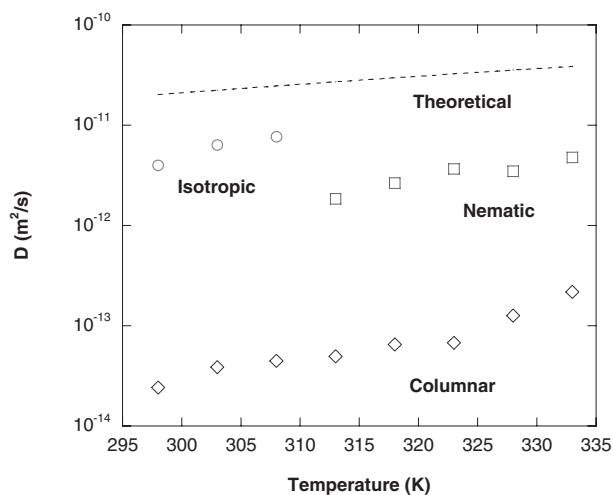


Figure 3. Plots of the self-diffusion coefficients of PBLG in 35% PBLG/1,4-dioxane solution against temperature with the theoretical calculated D values by Kirkwood theory using $d = 1.42$ nm, and the rod length $L = 69.4$ nm (dot line).

of each phase decrease by $^1\text{H } T_2$ in τ_1 period and decrease by T_1 in τ_2 period of PGStE ($\pi/2-\tau_1-\pi/2-\tau_2-\pi/2$ pulse(- τ_1 -echo)) method. Probably $^1\text{H } T_2$ and T_1 of PBLG in the columnar phase are much smaller than that in the nematic and isotropic phases. Consequently, the correct existent ratio of the columnar phase is much larger than the values listed in Table I. But it is possible to discuss qualitatively the existent ratio for each phase. The existent ratio of the columnar phase is much larger than that of the isotropic phase at low temperature. With increasing temperature, the existent ratio of the columnar phase decreases and that of the isotropic or nematic phases increases in 35% PBLG/1,4-dioxane.

Table II. Determined diffusion coefficients (D)^a of PBLG in 25% PBLG/1,4-dioxane solution at various temperature together with the fraction (f)^b of the corresponding diffusion coefficient components

Temperature/K	Diffusion coefficients/m ² /s		
	$D_{\text{isotropic}}(f_{\text{isotropic}})$	$D_{\text{nematic}}(f_{\text{nematic}})$	$D_{\text{columnar}}(f_{\text{columnar}})$
293	4.56×10^{-11} (0.97)		2.35×10^{-13} (0.03)
298	5.12×10^{-11} (0.99)		3.01×10^{-13} (0.01)
303	7.55×10^{-11} (0.99)		3.10×10^{-13} (0.01)
308	8.57×10^{-11} (0.98)	1.00×10^{-12} (0.02)	
313	8.46×10^{-11} (0.91)	1.91×10^{-12} (0.09)	
318	9.14×10^{-11} (0.91)	2.04×10^{-12} (0.09)	
323	1.08×10^{-10} (0.92)	1.89×10^{-12} (0.08)	
328	1.67×10^{-10} (0.92)	2.64×10^{-12} (0.08)	
333	1.70×10^{-10} (0.68)	3.34×10^{-12} (0.32)	
338	1.59×10^{-10} (0.80)	3.44×10^{-12} (0.20)	

^aThe experimental errors for the diffusion coefficient measurements are ca. 10%. ^bThe experimental errors for the fraction of the components are ca. 1%.

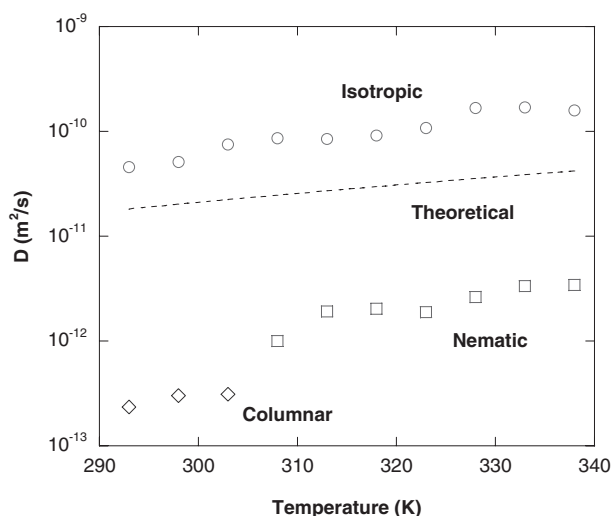


Figure 4. Plots of the self-diffusion coefficients of PBLG in 25% PBLG/1,4-dioxane solution against temperature with the theoretical calculated D values by Kirkwood theory using $d = 1.42$ nm, and the rod length $L = 69.4$ nm (dot line).

Diffusional Behavior of PBLG in 25% PBLG/1,4-dioxane Solution at Various Temperature

To obtain the self-diffusion coefficients (D) of PBLG in 25% 1,4-dioxane solution at wide temperature range, ¹H PGStE NMR measurements are performed from 293 to 338 K. The determined D values are listed in Table II together with the fraction of the corresponding diffusion coefficient components. Figure 4 shows the plots of the self-diffusion coefficients of molecules in 25% PBLG/1,4-dioxane solution against temperature. It was found that the faster diffusion coefficient gradually increases by increasing temperature, but that a transitional change of the slower diffusion coefficient exists between 303 K and 308 K.

From the phase diagram of Poly(γ -phenethyl L-gulutamete)/m-crezol system,¹⁸ it assumes that there exist the isotropic and columnar phases below 303 K and there exist the isotropic and nematic phases above 308 K in 25% PBLG/1,4-dioxane. Accordingly the faster diffusion component is

assigned to the isotropic phase at all temperature range. The slower diffusion component is assigned to the columnar phase below 303 K and to the nematic phase above 308 K. We indicate these assignments in Table II and Figure 4. The $D_{\text{isotropic}}$ are 4.56×10^{-11} to 1.59×10^{-10} m²/s, the D_{nematic} are 1.00 to 3.44×10^{-12} m²/s and the D_{columnar} are 2.35 to 3.10×10^{-13} m²/s, respectively. As we mentioned before, the fractions of the corresponding diffusion components in Table II are not correct existent ratios. Probably ¹H T_2 and T_1 of PBLG in the columnar and nematic phases are much smaller than that in the isotropic phase. Consequently, the correct existent ratio of the columnar and nematic phases is much larger than the values listed in Table II. But it is possible to discuss qualitatively the existent ratios of each phase. The existent ratio of the isotropic phase is much larger than that of the other phases at each temperature in 25% PBLG/1,4-dioxane.

Comparison with Theoretical Calculated D Values and Experimentally Obtained D Values

From Kirkwood theory,²⁴ the D of a rod-like molecule is shown as follow.

$$D_{\parallel} = \frac{k_B T \ln(L/d)}{2\pi\eta_s L} \quad (\text{in parallel to the rod direction}) \quad (3)$$

where k_B is the Boltzmann constant, T is the absolute temperature and η_s is the viscosity of the pure solvent. The η_s of 1,4-dioxane changes with temperature. (for example, 1.313 mPas at 293 K and 0.819 mPas at 323 K), and L is the rod length and d is the diameter of the rod. In this case, the PBLG molecules are parallel to the external magnetic field, so we can apply equation (3) for our systems. For a PBLG molecule, it is found that $d = 1.42$ nm, and $L = 69.4$ nm calculated by $DP = 463$.

In general equation (3) must not be applied to concentrated solutions because it only holds in dilute solutions for random-coiled polymer, because the polymer chains are entangled with each other in concentrated solution. But PBLG molecules take α_R -helical form and are treated as rod-like molecule in this case, and every PBLG molecule is parallel to the external magnetic field than there are no entanglements in the solution. So that we consider that it is possible to apply equation (3) to the PBLG molecule in this case. If one PBLG molecule independently diffuses, we can calculate the theoretical D value for one PBLG molecule from equation (3) using $d = 1.42$ nm, and the rod length $L = 69.4$ nm. We show the theoretical D values by dot line in Figures 3 and 4.

The D values in the isotropic phase in 25% PBLG/1,4-dioxane solution are a little bit larger than the theoretical D values for a PBLG molecule. It means a PBLG chain moves independently in the isotropic phase in 25% PBLG/1,4-dioxane solution. On the other hand, the D values in other phases in 25% PBLG/1,4-dioxane solution and that in all phases in 35% PBLG/1,4-dioxane solution are smaller than the theoretical D values for a PBLG molecule. It means several PBLG chains diffuse cooperatively in these phases. In the nematic phase, the only one-dimensional order exists along

with a molecular chain axis and there is no two-dimensional order. In this case, we consider that the PBLG molecules diffuse cooperatively as one *long rod* which containing the several PBLG chains forming a line. Using the experimentally obtained D values we can calculate the L_r which is the length of one *long rod* which containing the several PBLG chains forming a line from equation (3) with $d = 1.42$ nm. The L_r values are 1.51 to 3.05 μm in the nematic phase in 35% PBLG/1,4-dioxane solution and that are 2.45 to 5.47 μm in the nematic phase in 25% PBLG/1,4-dioxane solution. Since a PBLG chain length L is 69.4 nm, when we do not consider the spaces between chains, it means that 21.8 to 43.9 chains diffuse cooperatively keeping with one-dimensional order in the nematic phase in 35% PBLG/1,4-dioxane solution and 35.8 to 78.8 chains diffuse cooperatively also keeping with one-dimensional order in the nematic phase in PBLG/25% 1,4-dioxane solution.

In the columnar phase, there exists two-dimensional order,¹⁸ now we cannot deal with the PBLG chains forming a line by rod-like continuous long chains. But the smaller D values both in 35% PBLG/1,4-dioxane solution and in 25% PBLG/1,4-dioxane solution show that several PBLG chains diffuse also cooperatively keeping with two-dimensional order in the columnar phase.

CONCLUSIONS

The diffusion coefficient (D) of PBLG in the isotropic, nematic and columnar phases in concentrated solution is successfully determined by the field-gradient ^1H NMR methods. The D values of PBLG molecules in the isotropic phase in 25% PBLG/1,4-dioxane solution are a little bit larger than the identical calculated D values of a PBLG α_R -helical chain by the Kirkwood theory, which means PBLG chains move independently in the isotropic phase in 25% PBLG/1,4-dioxane solution. Whereas the D of PBLG molecules in other phases in 25% PBLG/1,4-dioxane solution and that in all phases in 35% 1,4-dioxane solution are smaller than the identical D of a PBLG α_R -helical chain. It means several PBLG chains move cooperatively in these phases. In the nematic phase, the PBLG molecules diffuse cooperatively as one *long rod* which containing the several PBLG chains forming a line. In the columnar phase, several PBLG chains diffuse also cooperatively keeping with two-dimensional order.

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