Side Chain Dynamics of Poly(γ -[κ - $^2\text{H}_1$]benzyl L-glutamate) and Poly(γ -[ζ - $^2\text{H}_2$]benzyl L-glutamate) by Solid State ^2H NMR

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ABSTRACT: In order to investigate side-chain dynamics of poly(γ -benzyl L-glutamate) in the solid state, ^2H NMR spectra and spin-lattice relaxation-time T_1 of poly(γ -[κ - $^2\text{H}_1$]benzyl L-glutamate) and poly(γ -[ζ - $^2\text{H}_2$]benzyl L-glutamate) were measured over a wide temperature range. Line shapes for both samples below room temperature showed static-like axially symmetric powder patterns whose quadrupolar splittings are slightly smaller than the rigid lattice values, and decreased gradually with temperature. This suggests the presence of a rapid, small-amplitude librational motions at both of κ - and ζ -positions. T_1 analysis for such librational motion gave the correlation time of the order of 10^{-11} – 10^{-12} s. The spectral intensity started to decrease gradually from 208 K with an increase in temperature, and a remarkable intensity loss was observed at around 323 K. With a further increase in temperature, the spectrum recovered its intensity, and the motional averaged singlet-like spectra were observed for both samples, indicating the presence of rapid, large amplitude motions for the side chains. The T_1 minimum was observed at 383 K. T_1 and line shapes were fairly well simulated by use of the three-site jump model assuming a Gaussian and a log-Gaussian distributions for the angle between the C- ^2H bond and the C_3 axis and the jump rate, respectively. The average jump rates are located on the relaxation map reported for poly(γ -benzyl L-glutamate). The motional mechanism at higher temperatures follows the WLF type.

KEY WORDS Poly(γ -benzyl L-glutamate) / Side Chain Dynamics / Solid State ^2H NMR / Spectral Simulation / Spin-Lattice Relaxation Time / Large Amplitude Motion / Librational Motion /

Poly(γ -benzyl L-glutamate) (PBLG), which takes an α -helical conformation in the solid state,^{1,2} has been studied by various techniques. It has been reported that the second moment of ^1H broadline NMR spectra for PBLG decreases rapidly at above room temperature.^{3–5} In the similar temperature region, a remarkable dielectric relaxation is observed and is well described by the WLF equation.^{6,7} It is also known that the specific volume-temperature curve for PBLG shows a break point at 291 K.⁸ These phenomena are caused by the side chains around helical cores which take pseudo hexagonal packing. That

is, the side-chain region shows a glass-like transition in the vicinity of room temperature and undergo rapid motions while the backbone keeps an α -helical conformation. Local internal motions along the side chain were recently investigated using nuclear magnetic relaxation measurements by CP/MAS ^{13}C NMR.⁹ The temperature dependence of $T_{1\rho}$ for each carbon in the side chain showed a minimum at around 350 K indicating that there are motions whose rates are of the order of 50 kHz in the side chains and that the motional process mainly corresponds to cooperative motion of whole side chains. It was suggested that side chain

Figure 1. Skeletal structure of PBLG side chain.

motions are highly anisotropic and/or the distribution of the correlation times is excessively broad.⁹

In this work, we investigated the side-chain dynamics of PBLG by means of the solid state ^2H NMR, in order to elucidate motional mode and rate of the side chain motions. ^2H NMR is a powerful technique of studying the local molecular motions.^{10–13} It enables us to discriminate clearly different types of motion and to measure correlation times over wide frequency range. Studying of the side chain dynamics gives us information about basic molecular motions of various biologically related polymers as well as the polymer with the long side chain.

The side-chain skeletal structure of PBLG is illustrated in Figure 1. ^2H NMR studies on deuterated phenyl group of PBDG showed that the phenyl ring undergoes rapid 180° flip-flop motion about the $\text{C}_\zeta\text{--C}_\eta$ axis and the flip-flop axis librates as well.¹⁴ In order to clarify motions of the side chain including the flip-flop axis, we measured ^2H NMR line-shapes and spin-lattice relaxation time T_1 of poly(γ -[κ - $^2\text{H}_1$]benzyl L-glutamate) and poly(γ -[ζ - $^2\text{H}_2$]benzyl L-glutamate). If the flip-flop motion occurs only about the $\text{C}_\zeta\text{--C}_\eta$ axis, the p -position of the phenyl ring would be unaffected by the flip-flop motion and therefore be static. ^2H NMR spectra of poly(γ -[κ - $^2\text{H}_1$]benzyl L-glutamate) would give direct information for the motion of flip-flop axis itself. This motion could be as well detected in poly(γ -[ζ - $^2\text{H}_2$]benzyl L-glutamate) because of having the common motional axis.

EXPERIMENTAL

Sample Preparation

Poly(γ -methyl L-glutamate) (PMLG) of $MW=83600$ was kindly supplied by Ajinomoto Co., Ltd. Poly(γ -[κ - $^2\text{H}_1$]benzyl L-glutamate) (PBLG- κd_1) and poly(γ -[ζ - $^2\text{H}_2$]benzyl L-glutamate) (PBLG- ζd_2) were obtained by the ester exchange reaction of PMLG with [p - $^2\text{H}_1$]benzyl alcohol and [α,α - $^2\text{H}_2$]benzyl alcohol, respectively. The extent of deuterium incorporation was determined to be greater than 97% by a high-resolution ^1H NMR spectroscopy at 500 MHz. All samples examined were films prepared by casting the chloroform solution at room temperature. For the NMR measurements, the films cut into small pieces were packed into the sample tube and sealed off after annealed at 373 K for 24 h *in vacuo*.

Measurements

The ^2H NMR measurements were performed on a Bruker MSL-200 spectrometer with a home-made 5-mm probe. The observed frequency was 30.7 MHz. All ^2H NMR spectra were obtained with the quadrupole echo pulse sequence ($90^\circ_x, \tau, 90^\circ_y, \tau$) with a delay time τ of 25 μs , a pulse of 1.6 to 1.8 μs and the dwell time of 0.2 μs . The quadrature-detected echo signal was left-shifted to the echo maximum and was Fourier transformed with a Lorentzian line broadening of 2 kHz. Spin-lattice relaxation time T_1 was obtained by the inversion-recovery quadrupole-echo pulse sequence ($180^\circ_x, \tau_d, 90^\circ_x, \tau, 90^\circ_y, \tau$). For both sequences, the recycle delay time was greater than $5T_1$. Simulation of deuterium powder line shapes was carried out on a Appolo DN10000 computer with a program MXQET.¹⁵

BRIEF DESCRIPTION ON THE SPECTRAL SIMULATION

It will be useful to outline first some of the basic features of ^2H NMR line shapes. The

deuteron is a spin $I=1$ nucleus possessing an electric quadrupole moment. The electric field gradient (EFG) in the deuteron interacts with the electric quadrupole moment to produce a shift in resonance frequency. The ^2H NMR frequencies of the symmetric line shape centered around the Larmor frequency ω_0 , which depend on the relative orientation of an external magnetic field B_0 and the EFG tensor, are given by¹⁶

$$\omega = \omega_0 \pm \frac{3}{8} \omega_Q (3 \cos^2 \beta - 1 - \eta \sin^2 \beta \cos 2\alpha) \quad (1)$$

where ω_Q is the quadrupole coupling constant, e^2qQ/h , η is the asymmetry parameter of the EFG tensor, and α and β are the spherical polar angles defining the orientation of B_0 in the EFG tensor principal axis system. In this work, we used $\omega_Q/2\pi = 170$ kHz and $\eta = 0.00$ for aliphatic deuterons, while $\omega_Q/2\pi = 180$ kHz and $\eta = 0.05$ for aromatic deuterons.^{17–19} For a powder sample (with C– ^2H bonds at various orientations β), the spectrum will be given by a properly weighted superposition of the signal corresponding to the each orientation, resulting in a typical pattern illustrated in Figure 2(a). The spectrum is symmetric about the Larmor frequency ω_0 , and the dominant characters of this line shape are two strong peaks disposed symmetrically about ω_0 and separated by $(3/4)\omega_Q$, and two extreme shoulders separated by $(3/2)\omega_Q$.¹⁸ The former corresponds to $\beta = 90^\circ$ orientation (referred as the “perpendicular components”) and the latter to $\beta = 0^\circ$ orientation (the “parallel components”). The separation $(3/4)\omega_Q$ is so called “quadrupolar splitting”.

^2H NMR spectra are profoundly influenced by motions.²⁰ It is possible to calculate the powder spectra in the presence of any type of motions by taking into account the angles, amplitudes, and rates involved, although in general this is difficult. However, in the “fast motion limit,” *i.e.*, when the frequency of the molecular motion is much larger than ω_Q , the

Figure 2. Calculated powder patterns for aliphatic C– ^2H bonds where $e^2qQ/h = 170$ kHz and $\eta = 0$. All spectra were added 2 kHz of Lorentzian line broadening. Jump rate is 10 MHz for spectra (b) and (c). (a) static powder pattern; (b) powder pattern averaged by fast three-site jumps about z_C axis ($\Theta = 45^\circ$); (c) powder pattern averaged by fast 8×12 equivalent sites jumps ($\Theta_i = 10^\circ, 20^\circ, 30^\circ, \dots, 80^\circ$, $\Phi_j = 30^\circ, 60^\circ, 90^\circ, \dots, 360^\circ$).

problem becomes much simpler. For fast motions, only the angular geometry is needed for calculations of spectra for well-defined motional models. In the fast motion limit, the ^2H NMR frequencies are given by²¹

$$\omega = \omega_0 \pm \frac{3}{4} \omega_Q \sum_{n=-2}^2 \rho_{2n} \overline{D_{n0}^{(2)}(\Omega_{\text{PL}})} \quad (2)$$

with $\rho_{20} = 1$, $\rho_{2\pm 1} = 0$ and $\rho_{2\pm 2} = -\eta/\sqrt{6}$. The $D_{ij}^{(2)}$ are the second-rank Wigner rotation matrix elements.²² Ω_{PL} are the Euler angles which give the orientation of the EFG tensor in the laboratory frame L whose z -axis is chosen parallel to the external magnetic field B_0 . The upper bar for $\overline{D_{n0}^{(2)}(\Omega_{\text{PL}})}$ denotes the time average over the motion. In order to perform such motional average, it is convenient to introduce the molecular frame C attached

to the molecules. We have then

$$\begin{aligned}\omega &= \omega_0 \pm \frac{3}{4} \omega_Q \sum_{n,a=-2}^2 \rho_{2n} \overline{D_{na}^{(2)}(\Omega_{PC}(t)) D_{a0}^{(2)}(\Omega_{CL})} \\ &= \omega_0 \pm \frac{3}{4} \omega_Q \sum_{n,a=-2}^2 \rho_{2n} e^{-in\alpha_{PC}(t)} \overline{d_{na}^{(2)}(\beta_{PC}(t))} \\ &\quad \times D_{a0}^{(2)}(\Omega_{CL})\end{aligned}\quad (3)$$

where Ω_{PC} relates the orientation of the EFG coordinate system to the C system, and $(\alpha_{PC}, \beta_{PC})$ are the spherical polar angles that define the orientation of EFG in the C system, $d_{na}^{(2)}(\beta)$ being the reduced rotation matrixes.²⁰ The later system is related to the L frame by Ω_{CL} . It is seen that the motional average is performed over Ω_{PC} while the numerical summation over Ω_{CL} to obtain the line shape depends on the orientational distribution of the molecules.

Consider a C-²H bond which can jump between three equivalent sites. The C-²H bond makes an angle Θ with respect to the z_C axis, which indicates z-axis in the C system, and the asymmetry parameter η is assumed to be zero for simplicity. In this case, the time average of ω is given by²³

$$\begin{aligned}\omega &= \omega_0 \pm \frac{3}{4} \omega_Q \left(\frac{3 \cos^2 \Theta - 1}{2} \right) \\ &\quad \times \left(\frac{3 \cos^2 \beta_{CL} - 1}{2} \right)\end{aligned}\quad (4)$$

where β_{CL} is the spherical polar angles defining the orientation of B_0 in the C system. With a powder sample, one would therefore expect a pattern analogous to a static (rigid-state) powder spectrum, but reduced along the frequency axis by the factor $(3 \cos^2 \Theta - 1)/2$, as illustrated in Figure 2(b).

Suppose a C-²H bond can jump among $M \times N$ ($N \geq 3$) positions with Θ_i , $i = 1, 2, \dots, M$ and $\Phi_j = 2\pi j/N$, $j = 1, 2, \dots, N$. (Θ_i, Φ_j) are the spherical polar angles that define the orientation of EFG tensor in the C system. If the motional mode is axially symmetric about z_C axis, *i.e.*, the populations of sites whose azimuthal angles are Φ_j , $p(\Phi_j)$, $j = 1, 2, \dots, N$,

are all equivalent, we have then

$$\begin{aligned}\omega &= \omega_0 \pm \frac{3}{4} \omega_Q \left(\sum_{i=1}^M p(\Theta_i) \frac{3 \cos^2 \Theta_i - 1}{2} \right) \\ &\quad \times \left(\frac{3 \cos^2 \beta_{CL} - 1}{2} \right)\end{aligned}\quad (5)$$

where $p(\Theta_i)$ is the population of sites whose polar angles are Θ_i . For the case that the C-²H bond jumps randomly between 8×12 equivalent sites ($\Theta_i = 10^\circ, 20^\circ, 30^\circ, \dots, 80^\circ$, $\Phi_j = 30^\circ, 60^\circ, 90^\circ, \dots, 360^\circ$), the calculated powder spectrum is shown in Figure 2(c). Using this model, one can obtain an axially symmetric powder pattern analogous to the above mentioned three-site case, but the reduction factor of spectrum width is different from that of three-site case. This fact suggests that a powder spectrum for multi-site jump model can be simulated by three-site jump model. In this case, we have

$$\frac{3 \cos^2 \Theta_0 - 1}{2} = \sum_{i=1}^M p(\Theta_i) \frac{3 \cos^2 \Theta_i - 1}{2} \quad (6)$$

where Θ_0 is the polar angle defining the orientation of the EFG tensor in the C system of three-site jump. In general the Θ_0 is not agree with the average value of polar angle for the multi-site jump model, $\langle \Theta \rangle = \sum_{i=1}^M p(\Theta_i) \Theta_i$, but $\langle \Theta \rangle$ can be approximated by Θ_0 when $\langle \Theta \rangle$ is around $\pi/4$.

If a C-²H bond diffuses uniformly over the surface of a cone having semiangle ϕ_C , we have

$$\begin{aligned}\omega &= \omega_0 \pm \frac{3}{4} \omega_Q \left(\frac{\cos \phi_C (1 + \cos \phi_C)}{2} \right) \\ &\quad \times \left(\frac{3 \cos^2 \beta_{CL} - 1}{2} \right).\end{aligned}\quad (7)$$

Then observed quadrupolar splittings, $\overline{\Delta\nu_Q}$, are given by²³

$$\overline{\Delta\nu_Q} = \Delta\nu_Q \frac{\cos \phi_C (1 + \cos \phi_C)}{2} \quad (8)$$

where $\Delta\nu_Q$ is a rigid lattice value, 128 kHz for both aromatic and aliphatic C-²H bond,

Figure 3. Experimental ^2H quadrupole echo spectra of poly(γ -[κ - $^2\text{H}_1$]benzyl L-glutamate) as a function of temperature. Figures in left-hand side of spectra indicate temperatures.

Figure 4. Experimental ^2H quadrupole echo spectra of poly(γ -[ζ - $^2\text{H}_2$]benzyl L-glutamate) as a function of temperature. Figures in left-hand side of spectra indicate temperatures.

utilizing the values described before.

RESULTS

The Temperature Dependence of Spectra

The temperature dependence of deuterium NMR spectra of PBLG- κd_1 and $-\zeta d_2$ are shown in Figures 3 and 4, respectively, and the temperature dependence of integrated intensities of PBLG- κd_1 and $-\zeta d_2$ are shown in Figure 5. The temperature range is from 168 to 403 K for both samples. The line shapes below about 208 K appear to be axially symmetric powder pattern for both samples,

Figure 5. Temperature dependence of integrated intensity for PBLG- κd_1 (○) and $-\zeta d_2$ (●). Intensity values of PBLG- κd_1 and $-\zeta d_2$ have been normalized by that at 191 and 168 K, respectively.

Figure 6. Temperature dependence of observed quadrupolar splittings $\overline{\Delta\nu_Q}$ for PBLG- κd_1 (○) and $-\zeta d_2$ (●).

indicating the absence of large amplitude motions on the ^2H NMR time scale. However, these line shapes are slightly different from complete static one. The parallel component is not clear and the perpendicular component is not sharp. The temperature dependence of quadrupolar splittings is shown in Figure 6. The values of quadrupolar splittings are slightly smaller than the rigid lattice values, and decrease gradually with increasing temperature. This suggests that there are rapid and small-amplitude librational motions at both sites, and that the amplitude of the motions increases with temperature. The signal intensity starts to decrease gradually from 208 K with an increase in temperature. Remarkable intensity loss is observed at around 323 K. With a further increase in temperature, the spectrum recovers its intensity. Above 353 K, the motionally averaged singlet-like spectra are observed, indicating the presence of rapid and large amplitude motions for the side chains. These results show that the side chains start to move with an increase in temperature and the temperature range about 323 K corresponds to the intermediate exchange region for these motions,¹⁰ in which the frequency of the motion is as high as the quadrupole coupling constant.

In addition to the intensity losses which occur in the intermediate exchange region, the spectra also show the τ dependence (data not shown). An increase in τ leads to a reduction of spectral intensity and characteristic line-shape changes. The τ dependence of spectra was observed around 323 K for both samples. However changes of intensity and shape of the spectra were small. This fact indicates that correlation times of the side-chain motion are distributed over a wide range.¹¹

Temperature Dependence of T_1

Spin-lattice relaxation time T_1 was measured in order to estimate the rate of side chain motions. The temperature dependence of T_1 is shown in Figure 7. With increasing tempera-

Figure 7. Temperature dependence of experimental (symbols) and calculated (lines) ^2H spin-lattice relaxation times T_1 . (○) Observed T_1 for PBLG- κd_1 , and (●) observed T_1 for PBLG- ζd_2 . Theoretical curves were calculated for three-site jump model, (---) for PBLG- κd_1 and (-·-) for PBLG- ζd_2 . (see the text in detail).

ture, T_1 for both samples decreases gradually and starts to decrease steeply from room temperature. T_1 minimum is observed at about 383 K for both samples. The minimum values of 4 and 6 ms are obtained for PBLG- κd_1 and $-\zeta d_2$, respectively. These facts indicate presence of motions of which rate are as high as Larmor frequency of 30.7 MHz at this temperature.¹⁶ Temperature dependence of T_1 is apparently divided into two parts at below and above room temperature. This suggests that T_1 values are governed by different motional mode of side chain such as librational motions and large amplitude motions in low and high temperature regions, respectively, as mentioned above for the temperature dependence of the spectra.

DISCUSSION

The Librational Motion in the Low Temperature Region

As mentioned above, rapid and small-amplitude librational motions exist in the side chain. The librational motions are probably brought about by the very small vibrations just like a superposition of the harmonic oscillation around a minimum of the potential of the

internal rotation. We tried to estimate the typical amplitude of librational motions of ϕ_C using eq 8. The value of ϕ_C increases with increasing temperature, being almost proportional to temperature. The least-squares lines fit to the results;

$$\text{for } \kappa d_1 \quad \phi_C = 5.97 + 0.0470 \times T \quad (9)$$

$$\text{for } \zeta d_2 \quad \phi_C = 10.3 + 0.0532 \times T. \quad (10)$$

In order to estimate the rate of the molecular motions, the theoretical T_1 values were calculated using models such as two-site jump, three-site jump, diffusion in a cone.^{21,24}

In the case of the jump between two equivalent sites separated by the jump angle α , T_1 is given as

$$\begin{aligned} \frac{1}{T_1} = & \frac{1}{8} \left(\frac{3\omega_Q}{4} \right)^2 A_1 \\ & \times \{g(\tau, \omega)[B_4 - (0.75B_1 - B_2)\cos 2\alpha] \\ & + g(\tau, 2\omega)[4B_5 - 4B_2\cos 2\alpha]\} \end{aligned} \quad (11)$$

where $g(\tau, \omega) = \tau/(1 + \omega^2\tau^2)$, $\tau = (2k)^{-1}$, and A_j , B_j are given in Table II of ref 21.

In the case of the jumps between three equivalent sites separated by the jump angle α , T_1 is given as

$$\begin{aligned} \frac{1}{T_1} = & \frac{1}{8} \left(\frac{3\omega_Q}{4} \right)^2 \\ & \times \{g(\tau, \omega)[A_1B_4 + A_2B_5 - 8A_3B_3\cos 3\alpha] \\ & + g(\tau, 2\omega)[4A_1B_5 + A_2B_6 - 8A_3B_3\cos 3\alpha]\} \end{aligned} \quad (12)$$

where $\tau = (3k)^{-1}$, and g , A_j , B_j are defined after eq 11.

If a C- ^2H bond diffuses freely, with diffusion coefficient D_w , in the cone of semiangle ϕ_C , then T_1 is given as²¹

$$\frac{1}{T_1} = \frac{1}{3} \left(\frac{3\omega_Q}{4} \right)^2 [J_1(\omega) + 4J_2(2\omega)]. \quad (13)$$

The spectral density functions in eq 13 are defined as

$$J_m(\omega) = 2 \int_0^\infty C_m(t) \cos(\omega t) dt. \quad (14)$$

Autocorrelation function $C_m(t)$ in eq 14 is given by²⁴

$$C_m(t) = \sum_{aa'=-2}^2 d_{ma}^{(2)}(\beta) d_{ma'}^{(2)}(\beta) e^{i(a-a')\alpha} C_{aa'}(t),$$

$$C_{aa'}(t) = \delta_{aa'} [G_a(\infty) + \{G_a(0) - G_a(\infty)\} e^{-t/\tau_{\text{eff}}^{(a)}}], \quad (15)$$

with approximation that the coupling tensor is axially symmetric ($\eta=0$). (α, β) is the spherical polar angle that define the orientation of the external field in the crystal-fixed coordinate system, and $d_{ma}^{(2)}(\beta)$ are the reduced Wigner rotation matrix elements. $G_a(\infty)$, $G_a(0)$, and $\tau_{\text{eff}}^{(a)}$ are given as functions of semiangle ϕ_C .

By using these models and eq 9 and 10 for the diffusion model, we tried to calculate T_1 values in the low temperature region. The correlation times at three temperatures are indicated in Table I. The correlation times for these models are scarcely different from each other, and of the order of 10^{-11} s. This fact indicates that the rate of the librational motions is so high to be in the fast limit region for line shapes.

For the line shape calculations, the librational motion was approximated by three-site jump model mentioned above for simplicity of calculations. Line shapes have been calculated at 168 K assuming equivalent populations for each site. The polar angles used in calculation are assumed to be the root mean square of ϕ_C derived from eq 9 and 10, and the values

Table I. Correlation times calculated for PBLG- κd_1 and $-\zeta d_2$ ^a

Temp K	PBLG- κd_1			PBLG- ζd_2		
	2-Site jump	3-Site jump	Diffusion in a cone/s	2-Site jump	3-Site jump	Diffusion in a cone/s
	s	s		s	s	
298	1.98×10^{-11}	1.77×10^{-11}	2.02×10^{-11}	1.87×10^{-11}	1.67×10^{-11}	1.65×10^{-11}
248	2.00×10^{-11}	1.79×10^{-11}	1.75×10^{-11}	1.89×10^{-11}	1.68×10^{-11}	1.49×10^{-11}
198	2.02×10^{-11}	1.81×10^{-11}	1.53×10^{-11}	1.91×10^{-11}	1.70×10^{-11}	1.32×10^{-11}

^a Correlation times were obtained by fitting the experimental T_1 values as described in the text.

Figure 8. Simulated ^2H quadrupole echo spectra of (a) and (b) PBLG- κd_1 , (c) and (d) $-\zeta d_2$ at 168 K for (a) and (c) no distribution for ϕ_0 , (b) and (d) Gaussian distribution. Averaged value $\langle \phi_0 \rangle$ are 10° and 14° for PBLG- κd_1 and $-\zeta d_2$, respectively. Standard deviation $\Delta \phi_0$ is 5° for both samples.

are 10° and 14° for PBLG- κd_1 and $-\zeta d_2$, respectively. The results are shown in Figures 8(a) and (c). Though these calculated spectra have the same quadrupolar splitting as for observed ones, their line shapes are slightly different from observed ones. These are improved by considering the distribution of the amplitude of the librational motions, since the side chains take various conformations due to the disordered structure. The calculated spectra are shown in Figures 8(b) and (d) assuming the Gaussian distribution for ϕ_0 . They are in very good agreement with the observed.

Large Scale Motion in the High Temperature Region

High temperature spectra were calculated in

order to investigate the mechanism of large amplitude motions related to the side-chain conformational change. It is considered that rapid and large amplitude motions of the side chain are caused by multibond internal rotations at high temperatures.⁹ In order to see such a situation, allowed conformations which occur during jump motion were calculated for a single side chain. Projections of a side chain on the plane perpendicular to the helix axis is shown in Figure 9(a). This calculation was carried out under following assumptions²⁵; (1) each of the six internal rotation angles ($\chi_1, \chi_2, \chi_3, \chi_4, \chi_5, \chi_6$) takes three rotational isomeric states (*t, g, g'*), and (2) any atom of a side chain cannot approach within the van der Waals radius of each atom.

Figure 9. (a) Allowed conformations of PBLG side chain (projection of a single side chain along the helix axis). The large amplitude motions at high temperature were caused by multibond internal rotations. (b) Distribution for $C_{\kappa-2}H$ bond vectors of the side chain in (a) around the average direction. (c) Three-site jump model whose polar angle is θ , approximated for multi-site jump motion in (b) around the average direction.

Directions of a $\text{C}_\kappa\text{-}^2\text{H}$ bond or a $\text{C}_\zeta\text{-}^2\text{H}$ bond are shown in Figure 9(b). The $\text{C}\text{-}^2\text{H}$ bond vectors distributed nearly uniformly about the average direction. Motion of these bonds will occur by jumps among such directions which are distributed around the average direction, just like a cone. Therefore, in this case, use of the diffusion in a cone is a good approximation of multi-site jump model for the spectral simulation. However, here, equally populated three-site jump model is adopted for simplicity of calculation as discussed above (Figure 9(c)). The jump rate is supposed to be fast enough to give the T_1 minimum at 383 K and, therefore, in the fast motional limit for spectral simulation. The librational motion with small amplitude will exist at high temperatures as well as low temperatures, and was taken into account by the three-site jump model, as described below, in T_1 calculation. In this model the $\text{C}\text{-}^2\text{H}$ bonds undergo motions superimposed with two independent three-site jumps. For the librational motions, a single value of ϕ_0 was used for the simplicity of calculation. The jump rate of the librational motions was fixed at $1 \times 10^{11} \text{ s}^{-1}$. For the large amplitude motions, we assumed a log-Gaussian and a Gaussian distributions for the jump rate and the angle θ defining the angle between the

three fold axis and the $\text{C}\text{-}^2\text{H}$ bond, respectively.

The calculated spectra at 383 K where T_1 minimum appears are shown in Figures 10(a) and 11(a). The average angle $\langle\theta\rangle$ is obtained to be 47° and 49° for PBLG- κd_1 and $-\zeta d_2$, respectively. The standard deviation of θ , $\Delta\theta$, is obtained to be 3.8° for both samples. The fact that these parameters are almost same for both samples is reasonable, because the $\text{C}_\kappa\text{-}^2\text{H}$ bond and the $\text{C}_\zeta\text{-}^2\text{H}$ bond attach to the common motional axis (the $\text{O}_\varepsilon\text{-C}_\zeta$ axis). The average jump rate is estimated to be $2\pi\nu_0/3 = 64(\text{MHz})$ at 383 K from the observed T_1 value, and the distribution width of the jump rate is 1.3 in log scale.

The calculated spectra for several temperatures using the same model are shown in Figures 10(c), and 11(c), (e), and (g). The fitting parameters obtained are shown in Tables II and III. In this temperature region, the three-site jump model is found to be not always a good approximation in terms of jump rate. Although the jump rate is not sufficiently fast to be in the fast motional limit of line shapes, the spectra could be fairly fitted with observed ones. This is ascribable to facts that the calculated spectra are superposition of each spectrum having each rate. The resultant line shape is governed by line shapes having fast

Figure 10. ^2H quadrupole echo spectra of PBLG- κd_1 ; (a) and (c) are simulated line shapes corresponding to the experimentals at 383 K (b) and 333 K (d), respectively.

Figure 11. ^2H quadrupole echo spectra of PBLG- ζd_2 ; (a), (c), (e) and (g) are simulated line shapes corresponding to the experimentals at 383 K (b), 333 K (d), 329 K (f), and 327 K (h), respectively.

Table II. Parameters for calculation with the motional model at high temperatures in PBLG- κd_1

Temp	$\langle\theta\rangle$	$\Delta\theta$	$\langle S_\theta\rangle$	$\langle k\rangle$	$\Delta\log k$
K	deg	deg		kHz	kHz
383	47	3.8	0.21	64000	1.3
333	38	13	0.42	35	1.3

Table III. Parameters for calculation with the motional model at high temperatures in PBLG- ζd_2

Temp	$\langle\theta\rangle$	$\Delta\theta$	$\langle S_\theta\rangle$	$\langle k\rangle$	$\Delta\log k$
K	deg	deg		kHz	kHz
383	49	3.8	0.15	64000	1.3
333	38	13	0.42	20	1.3
329	38	13	0.42	15	1.3
327	38	13	0.42	8	1.3

or slow enough rate whose intensities are by far higher than that of intermediate region. It is found that the average values of θ and the standard deviations change drastically at around 340 K as a consequence of glass-like transition in the side chain region.⁸

Order parameter $\langle S_\theta \rangle$ which corresponds to the ensemble averaged orientational function for C- ^2H bonds is defined as

$$\langle S_\theta \rangle = \left\langle \frac{3 \cos^2 \theta - 1}{2} \right\rangle \quad (16)$$

and bracket $\langle \rangle$ denotes ensemble average. The values of $\langle S_\theta \rangle$ calculated using the parameters derived from line-shape calculations in the high temperature are shown in Tables II and III.

$\langle S_\theta \rangle$ values at 383 K are obtained to be 0.21 and 0.15 for PBLG- κd_1 and $-\zeta d_2$ respectively, while those at below 333 K are 0.42 for both samples. This shows that above the glass-like transition temperature, raising temperature gives increase in the amplitude of the side chain motions.

The theoretical temperature dependence of T_1 for three-site jump are calculated using the parameters derived from line-shape calculations at 383 K which is the temperature taking a T_1 minimum, and are shown in Figure 7. Temperature dependence of correlation time follows WLF type (see below).⁶ At around the minimum, the calculated values are in very good agreement with the experimental ones, especially for PBLG- κd_1 . This indicates that temperature dependence of T_1 for both samples in high temperature region is originated in the large amplitude motions caused by the multibond internal rotations.

The jump rate obtained from the line shape calculations at the higher temperatures are plotted on the relaxation map obtained from other experimental methods in Figure 12. The

Figure 12. Relaxation map for PBLG side chain motion from dielectric relaxations ($\diamond$), dynamic mechanical measurements ($\times$), ^{13}C NMR ($T_{1\rho}$ of phenyl carbon)⁹ ($\times$), ^2H line shape simulations of PBLG- κd_1 ($\bullet$), and of $-\zeta d_2$ ($\square$).

points for the mechanical and dielectric relaxations are corresponding to the process for the large scale side-chain motions (β -process).⁹ The present results from the ^2H NMR experiments are located on the curve obtained from other relaxation experiments. This fact shows that the motional mechanism at high temperature follows the WLF type.

CONCLUSION

We have shown the presence of mainly two motional modes in the PBLG side chain. One is the rapid and small-amplitude librational motion, and the other is the large amplitude motion caused by the multibond internal rotations. The complex molecular dynamics of the side chain in disordered state is characterized by distributions of motional amplitudes and of correlation times. The librational motions are brought about by the very small vibrations just like a superposition of the harmonic oscillation around a minimum of the potential of the internal rotation. Such motion makes the observed quadrupolar splitting slightly smaller and affects remarkably the T_1 values below room temperature. Motional range of the librational motion is about $\pm 10^\circ$ and it has a distribution whose width is 5° at 168 K. The correlation time of the librational motion is of the order of 10^{-11} to 10^{-12} s in the temperature range measured. The side chains of PBLG undergo rapid and large amplitude motions whose rate are of the order of more than 10 MHz above 373 K with the distribution width of 1.3 in log scale in high temperatures. The averaged rates estimated from present ^2H NMR experiments are located on the relaxation map obtained from other experimental methods. The motional mechanism at higher temperatures follows the WLF type.

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