Side Chain Motion and Structure of Racemic Poly(γ -benzyl glutamate) as Studied by Solid State High-Resolution ^{13}C Nuclear Magnetic Resonance

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ABSTRACT: The side chain motion and structure of the racemic mixture of poly(γ -benzyl glutamate) (PBDLG) in the solid state were studied by means of the cross polarization/magic angle spinning (CP/MAS) ^{13}C NMR. C_β and C_γ regions of NMR spectra for PBDLG in the stacked state of phenyl rings exhibit a remarkable difference from those for poly(γ -benzyl L-glutamate) (PBLG) with no side chain stacking. Use of γ - d_2 PBDLG, deuterated at C_γ , showed that the upfield shift occurs in a part of γ -carbons. It was suggested that this new peak arises from the regular side chain conformation with phenyl ring stacking. The measurements of ^{13}C $T_{1\rho}$, spin-lattice relaxation time in the rotating frame, indicated that both the side chain and main chain motions are restricted in the stacked state. However, it is noticeable that such effect of stacking is especially remarkable for C_γ rather than phenyl ring. This means that phenyl rings have a rather high mobility even in the stacked state.

KEY WORDS Racemic Poly(γ -benzyl glutamate) / Solid State ^{13}C NMR / Spin-Lattice Relaxation in Rotating Frame / Side Chain Motion / Phenyl Ring Stacking /

Side chain motions of poly(α -amino acid)s have been studied by many workers.^{1–3} It has been reported that the mixture of two kinds of α -helical polymers, each having an opposite screw sense, shows different solid state properties from those of each polymer.⁴ Racemic mixture of poly(γ -benzyl glutamate) (PBDLG) which consists of poly(γ -benzyl L-glutamate) (PBLG) and poly(γ -benzyl D-glutamate) (PBDG) has been investigated extensively.^{5–10} X-Ray diffraction measurements have shown an 'extra' meridional reflection of 10.5 Å, which is not observed in PBLG or PBDG.⁷ Squire and Elliott proposed the model that side chain phenyl rings in PBDLG stack regularly.⁸ Uematsu *et al.* reported that the extra reflection peak disappears around 90°C and ascribed this phenomenon to the breakdown of 'stacking structure'.⁹ Takahashi

*et al.*⁶ and Matsushima *et al.*¹⁰ have made dielectric relaxation and X-ray diffraction measurements, respectively and discussed this phenomenon as the order-disorder transition. It is well known that ^{13}C cross polarization/magic angle spinning (CP/MAS) NMR is one of the most powerful methods for studying local structures and mobilities of polymers in the solid state. In this work, to obtain further detailed information about local structure and dynamics of the side chain in PBDLG, ^{13}C CP/MAS measurements have been carried out.

EXPERIMENTAL

Samples

PBLG and PBDG were prepared by the conventional NCA method. PBDLG was ob-

tained by casting the chloroform solution of 1:1 mixture of PBLG and PBDG. For the NMR measurements, these films were annealed at 130°C for 12 hours in vacuum. Differential scanning calorimetry (DSC) measurements of this sample showed an endothermic peak at 90°C, indicating that side chains of PBDLG take the 'stacking structure' at room temperature.¹¹ γ - d_2 PBLG and PBDG, where γ -carbon in the side chain is deuterated, were prepared by the previously reported procedures¹² to obtain the γ -deuterated sample of PBDLG, γ - d_2 PBDLG.

Measurements

^{13}C CP/MAS NMR measurements were performed by Bruker MSL-200 spectrometer (^{13}C 50 MHz) with the MAS rate of 3.8 kHz above room temperature. Chemical shifts were calibrated through the external adamantane (29.5 ppm relative to TMS). ^{13}C spin-lattice relaxation time in the rotating frame, $T_{1\rho}$, was measured with varying the spin-lock frequency from 40 kHz to 73.5 kHz using the pulse sequences in Figure 1(a). Since there are no ^1H dipolar interactions from the directly attached γ -protons for γ - d_2 PBDLG, the ^1H local field at C_γ is considerably smaller compared to that at C_β . The cross relaxation time when protons are spin-locked, T_{CH} , is dependent on the magnitude of the dipole interaction, and expressed as follows.¹³

$$1/T_{\text{CH}} = (1/2)M_{\text{CH}}^{(2)}J_X^{(0)} \quad (1)$$

Where $M_{\text{CH}}^{(2)}$ is the second moment of ^{13}C - ^1H dipole interaction, and $J_X^{(0)}$ is the spectral density of spin fluctuations. C_γ of γ - d_2 sample is expected to have relatively longer T_{CH} because of the smaller $M_{\text{CH}}^{(2)}$ than C_β due to the lack of directly attached protons. This means that C_γ needs a longer time to obtain an enough ^{13}C magnetization by cross polarization. Further, the transverse relaxation time, T_2 , of C_γ in γ - d_2 sample is longer than that of C_β due to the smaller local field. On the basis of these facts, it is possible

Figure 1. Pulse sequences used for ^{13}C CP/MAS NMR measurements. (a) Pulse sequence to measure the ^{13}C $T_{1\rho}$ with CP, (b) CP/MAS sequence with a short CP time used for C_β of γ - d_2 PBDLG, and (c) CP/MAS sequence with a delay time before acquisition used for C_γ of γ - d_2 PBDLG. Where CP, cross polarization; DD, dipole decoupling; FID, free induction decay; SL, spin locking.

to obtain selectively C_β peak using the pulse sequence shown in Figure 1(b) with a rather short CP time during which C_γ cannot attain the enough magnetization. On the other hand, C_γ peaks can be obtained selectively by the pulse sequence in Figure 1(c) with a delay time before acquisition, since during this time C_β magnetization is died away with a shorter time constant T_2 than that of C_γ .

RESULTS AND DISCUSSION

In Figure 2, ^{13}C CP/MAS NMR spectra of PBLG and PBDLG are shown. There is no difference of the chemical shifts for all peaks including phenyl ring between both samples except for C_β and C_γ peaks. For PBDLG,

Figure 2. ^{13}C CP/MAS NMR spectra (50 MHz) of PBLG and PBDLG below and above the break down temperature of stacking structure. MAS rate = 3850 Hz; CP time = 1 ms; repetition time = 5 s.

these peaks are not resolved at 30°C (stacked state) differing from PBLG, whereas at 160°C (unstacked state), two peaks are well resolved and spectrum is similar to that of PBLG at 120°C. These results indicate that 'stacking structure' affected the side chain conformation at portions near the helical backbone. It is of interest that phenyl peaks show no difference between the stacked and unstacked states.

^{13}C CP/MAS NMR spectra of PBLG, PBDG, and their addition were compared in order to see that the unresolved peaks of C_β and C_γ are caused by the 'stacking structure'. No essential differences are seen among these spectra, indicating that the unresolved peaks of C_β and C_γ are not caused by the inherent difference of PBDG and PBLG.

Temperature dependence of C_β and C_γ peaks are shown in Figure 3. The line shape of these peaks changes largely with increasing

Figure 3. Temperature dependence of ^{13}C CP/MAS NMR spectra of PBDLG (C_β and C_γ region).

temperature, and there exists a new peak as shown with an arrow in the spectrum at 110°C. However, at the higher temperature, the new peak disappears. The chemical shift of this new peak is clearly different from that of C_β and C_γ in PBLG.

It seems important at this stage to clarify the origin of the new peak. For this purpose, γ -deuterated samples, γ - d_2 PBDLG, were used. In Figure 4, C_β and C_γ spectra for γ - d_2 PBDLG are shown. The CP/MAS NMR spectrum with the maximum S/N condition by the pulse sequence in Figure 1(b) is shown in Figure 4(a), where contributions from both C_γ and C_β peaks are seen, whereas, in Figures

Figure 4. ^{13}C CP/MAS NMR spectra (50 MHz) of γ -deuterated PBDLG (C_β and C_γ region).

4(b) and (c), only C_β and C_γ peaks are seen, respectively. The line shape of the spectrum in Figure 4(b) is symmetric and can be fitted by a single Lorentz curve and the chemical shift is similar to that of C_β in PBLG (26.5 ppm). On the other hand, the spectrum in Figure 4(c) measured by the pulse sequence of Figure 1(c) is not symmetric and can be fitted by the superposition of two Lorentz curves. The chemical shift values of these curves are 30.55 ppm (similar to that of C_γ) and 28.0 ppm (middle of C_β and C_γ), respectively. These facts indicate that the origin of the new peak is C_γ . It is suggested that around C_γ portion, the side chain in the stacked state takes a certain conformation which has not been reported, although the definite model is not

Figure 5. Spin-lock frequency, f_1 , dependence of ^{13}C $T_{1\rho}$ for each carbon of PBDLG. Δ , C_α ; $\blacklozenge$, C_β and C_γ ; $\circ$, C_{bz} ; $\cdot$, C_{phe} ; $\times$, $\text{C}_{\text{phe}}^{\#}$; $+$, CO (ester); Y, CO (amide). The solid line is proportional to f_1^2 and the broken line to $\exp(\tau_D^2 \pi f_1)$, where $\tau_D = 33.6 \mu\text{s}$.^{16,17}

proposed at the present stage. Furthermore, present results show that the side chains with the similar conformation to that in PBLG also exist, since in the stacked state, C_γ peak which has similar chemical shift to that of PBLG is also observed. This is in agreement with the suggestion by Squire and Elliott; some of side chains can not take the 'stacking structure' because of geometric structure of α -helix.⁸

To investigate the motional state of side chains in the stacked state, ^{13}C spin-lattice relaxation time in the rotating frame, $T_{1\rho}$, was measured. It is well known that $T_{1\rho}$ measurement with ^{13}C CP/MAS NMR is potentially the most powerful tool for studying the local mobility of polymers. However, in actual case, because of the existence of ^{13}C - ^1H heteronuclear mutual spin flip-flop, the ^{13}C magnetization relaxes via two path ways with 'true' spin-lattice relaxation time in the rotating frame, $T_{1\rho}$, and cross relaxation time, T_{CH} . Thus, the experimentally observed relaxation

time $T_{1\rho}^*$ is expressed as follows.¹⁴

$$1/T_{1\rho}^{**} = 1/T_{1\rho} + 1/T_{\text{CH}} \quad (2)$$

Therefore, it is important to know if 'observed' relaxation time, $T_{1\rho}^*$, reflects the molecular motion. For PBLG, it was reported that $T_{1\rho}^*$ mainly reflects $T_{1\rho}$ process.¹⁵ To

confirm this for PBDLG, dependence of $T_{1\rho}$ on the ^{13}C spin-lock frequency, f_1 , was measured, because $T_{1\rho}$ and T_{CH} have very different f_1 dependences. Since $T_{1\rho}$ is proportional to $1 + (2\pi f_1)^2 \tau^2$, where τ is the correlation time, $T_{1\rho}$ depends on the second power of f_1 at most (slow motion limit). On the

Figure 6. Temperature dependence of ^{13}C $T_{1\rho}$ for C_γ (a), C_{phe} (b), and C_α (c) of PBDLG (■) and PBLG (◇).

other hand, T_{CH} depends on f_1 as $\exp(2\pi\tau_D f_1)$, where τ_D is the dipole fluctuation time and given by^{16,17}

$$\tau_D = (9/M_{CH}^{(2)})^{1/2} \quad (3)$$

In Figure 5, the logarithm of $T_{1\rho}^{**}$ for each carbon of PBDLG at room temperature is plotted against the spin-lock frequency f_1 . The solid and broken lines are proportional to logarithms of f_1^2 and $\exp(2\pi\tau_D f_1)$, respectively. The slope of $T_{1\rho}^{**}$ for each carbon is much smaller than that of the broken line. This indicates that $T_{1\rho}^{**}$ is mainly contributed from the $T_{1\rho}$ process which reflects molecular motion. Therefore, hereafter, $T_{1\rho}^{**}$ is regarded as $T_{1\rho}$. For C_β and C_γ , f_1 dependence of $T_{1\rho}$ is slightly larger than for other carbons in side chains. This is characteristics for PBDLG in the stacked state, and may be interpreted by the increase of the contribution from T_{CH} due to the restricted side chain motion at around C_β and C_γ .

Temperature dependence of $T_{1\rho}$ for C_γ with $f_1 = 73.5$ kHz is shown in Figure 6(a) together with the results for PBLG. In the stacked state, $T_{1\rho}$ for PBDLG is considerably larger than that of PBLG and slightly decreases with increasing temperature, whereas the $T_{1\rho}$ for PBLG changes pronouncedly and shows the minimum at around 2.7 ($10^3 T^{-1}$), the relation $2\pi f_1 \tau \sim 1$ is realized. These facts indicate that the correlation time of ^{13}C in PBDLG is in the range much longer than $1/2\pi f_1$ due to highly restricted side chain motion by the 'stacking structure'. However, around $T \sim 100^\circ C$ (broken line in Figure 6(a), hereafter abbreviated as T_B), where the breakdown of the 'stacking structure' occurs, $T_{1\rho}$ for PBDLG suddenly decreases to the similar value to that for PBLG. Above that temperature, there is no significant difference between two samples, indicating that the motional state of the side chains in PBDLG is similar to that of PBLG. In the latter, rapid motion of whole side chains occurs above room temperature.^{1,15} These phenomena are fairly well

corresponding to the results of dielectric relaxation measurements.⁶

Temperature dependence of $T_{1\rho}$ for C_{phe} are shown in Figure 6(b). For PBLG, $T_{1\rho}$ shows the minimum at around 2.9 ($10^3 T^{-1}$), where ^{13}C - 1H pairs of phenyl ring have the correlation time of the order of $(2\pi f_1)^{-1} \sim 2.2 \times 10^{-6}$ s. This temperature is very close to the curve on the relaxation map corresponding to the motion of whole side chain.^{1,6,15} Therefore, phenyl rings undergo rapid motion above room temperature. For PBDLG, $T_{1\rho}$ has larger values than that for PBLG below T_B , where 'stacking structure' is formed. This implies that motion of phenyl ring is restricted due to the side chain stacking. However, motional states of phenyl ring for PBLG and PBDLG are similar above T_B , since $T_{1\rho}$ values are similar. The value of $T_{1\rho}$ minimum which appears at 2.75 ($10^3 T^{-1}$) is rather larger than that at 2.9 ($10^3 T^{-1}$) for PBLG and this cannot be reasonably interpreted. As seen in C_γ (Figure 6(a)), abrupt change of $T_{1\rho}$ is expected for C_{phe} at around T_B . However, such change is not observed. It is considered that the $T_{1\rho}$ minimum at around T_B for PBDLG appears apparently as a consequence that the temperature dependence of $T_{1\rho}$ changes from that of PBDLG to PBLG. Furthermore, the fact that values of $T_{1\rho}$ are not greatly different between PBLG and PBDLG as seen for C_γ below T_B may imply that phenyl rings in PBDLG undergo rather rapid motion in spite of the existence of the 'stacking structure'.

It is noticeable that the effects of the 'stacking structure' on the molecular motion are more clearly seen in the side chain portion which is near to the backbone. Present results indicate that even in the stacked state phenyl rings have a considerably higher degree of motional freedom than that expected from the model believed here-to-fore such as 'static' regular stacking.

Temperature dependence of $T_{1\rho}$ for C_α in the main chain is shown in Figure 6(c). For

PBLG, $T_{1\rho}$ is larger than that of side chain and decreases gradually with increasing temperature, indicating that the molecular motion is highly restricted compared with that of side chain. For PBDLG, $T_{1\rho}$ is further longer than that for PBLG. This means that presumably the small amplitude oscillation^{18,19} of α -helical main chain is much restricted by the side chain stacking below T_B . A sudden decrease is seen at the breakdown temperature T_B . Above T_B , no difference is observed between PBLG and PBDLG, indicating that mobility of the main chain in both polymer is similar each other. Such effect on the motion of the rigid α -helical main chain may be closely related to the fact that the side chain portion near the main chain is pronouncedly restricted by the 'stacking structure'. More detailed analysis and discussion on the motional mechanisms, including those for the side chains, will be published elsewhere in near future.

CONCLUSION

In the stacked state of PBDLG, a part of γ -carbons have a different chemical shift, new peak, from that of PBLG. At the higher temperature (unstacked state), such 'new' peak disappears. This suggests that the portion of side chain near main chain takes a conformation characteristics of the 'stacking structure'. On the other hand, chemical shifts of phenyl ring carbons did not show difference between stacked and unstacked states. The mobilities of both of side chain and α -helical main chain are restricted in the stacked state. It is noticeable that such effects of stacking on the side chain motion are more remarkable in C_γ rather than C_{phe} . At the breakdown temperature of the stacking, ^{13}C $T_{1\rho}$ changes discontinuously and the mobility of the side chains and the main chain increases. In the unstacked state of PBDLG, the motional state

is essentially same as that of PBLG.

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