SHORT COMMUNICATIONS

# Methyl Group Dynamics in Poly($\beta$-methyl L-aspartate) by Solid State $^2$H NMR

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Solid-state $^2$H NMR spectroscopy is a powerful method of investigating molecular dynamics in the solid state.[1,2] The major advantage of this method derives from the facts that a quadrupole coupling is the dominant interaction and the electric field gradient (EFG) tensor is usually axially symmetric for a deuteron bonded to carbon, with the unique axis along the C–$^2$H bond. These features permits one to study internal dynamics spanning a range of rates from $10^4$ to $10^{12}$ s$^{-1}$ from line shapes and relaxation times of $^2$H NMR spectra in a relatively direct manner.[1,2] In the case of a methyl group, rapid 3-fold rotation yields an averaged EFG whose symmetry axis is along the rotation axis ($C_3$ axis), and the corresponding line shape is axially symmetric with a quadrupole splitting of about 40 kHz.[3,4]

Poly(aspartate esters) take on various chain conformations in solid as well as in solution, depending on the temperature, the solvent, and the intrinsic properties of the different ester side chains. Their conformations were well characterized with various methods.[5-14] However, there is few works on the side chain motions about these polypeptides,[15,16] compared with poly(glutamate esters).[17] The broad-line pro-

ton NMR study showed that there is considerable mobility of the side chain as well as the methyl rotation at above room temperature in poly($\beta$-methyl L-aspartate) (PMLA).[15] PMLA has the left-handed $\omega$ helix in solution and film.[5,6] In this work, we present a quantitative examination of the side chain motions of PMLA with solid-state $^2$H NMR line-shape and spin-lattice relaxation of a deuterio-methyl PMLA.

## EXPERIMENTAL

Poly($\beta$-[methyl-$^2$H$_3$] L-aspartate) (PMLA-$d_3$) was prepared by ester exchange reaction of poly($\beta$-benzyl L-aspartate) (MW = 58000) purchased from Sigma, in methylene chloride with methanol-$d_4$ (99.7%) from Merck Isotopes. The extent of the exchange was estimated to be over 95%, judged from high-resolution $^1$H NMR spectroscopy at 500 MHz. Solid state $^2$H NMR measurements were recorded at 30.7 MHz on a Bruker MSL-200 spectrometer with a home-made 5-mm horizontal solenoid coil. Spectra were obtained by the quadrupole pulse sequence with the 90 degree pulse of 1.7 $\mu$s or less, the delay between pulses of 25 $\mu$s, and the dwell time of 0.5 $\mu$s. Spin-lattice relaxation time

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$(T_1)$ was determined with an inversion-recovery quadrupole pulse-sequence. Simulations of the $^2$H NMR line shapes were carried out with a program described by Greenfield and co-workers.[18]

## RESULTS AND DISCUSSION

Figure 1 shows the temperature dependence of deuterium NMR spectra for PMLA-$d_3$ from $-125°$C to $119°$C. The spectrum at $-125°$C is a well-defined Pake doublet with the quadrupole splitting of 38 kHz, having the asymmetry parameter of nearly zero. Such an axially symmetric powder pattern with an effective quadrupole coupling constant of 50.7 kHz is consistent with fast reorientation of the methyl group. There is no substantial change in line shapes from $-125°$ to $50°$C. Slight change in line shapes are observed at higher temperatures above 50°C; the signal-to-noise ratio of a spectrum becomes worse and the integrated intensity of a spectrum decreases. These results suggest the presence of motion(s) of the rate between $10^4$ and $10^6\,\mathrm{s}^{-1}$, that is, in the intermediate exchange.[4,19,20]

The quadrupole splitting decreases from 38 to 34 kHz on going from $-125°$C to $119°$C. The observed quadrupole splitting is slightly smaller than the theoretically predicted value of 41.7 kHz in the case of extremely fast reorientation about $C_3$ axis, assuming the quadrupole coupling constant of 167 kHz for the deuterio-methyl group.[4] This suggests the presence of the additional rapid ($< 10^{-11}\,\mathrm{s}$) librational motions of the $C_3$ axis, in addition to the $C_3$ rotation. This small decrease in quadruple splitting may be accounted for by such motions of the $C_3$ axis within a cone of semiangles between 20° to 30°.[3] Since large-amplitude motions having the rate faster than $10^{-6}\,\mathrm{s}$ would result in a significant narrowing of the line shape because of averaging of the EFG tensor, these would be excluded.

Inspection of the inversion-recovery $^2$H NMR spectra revealed that the parallel com-
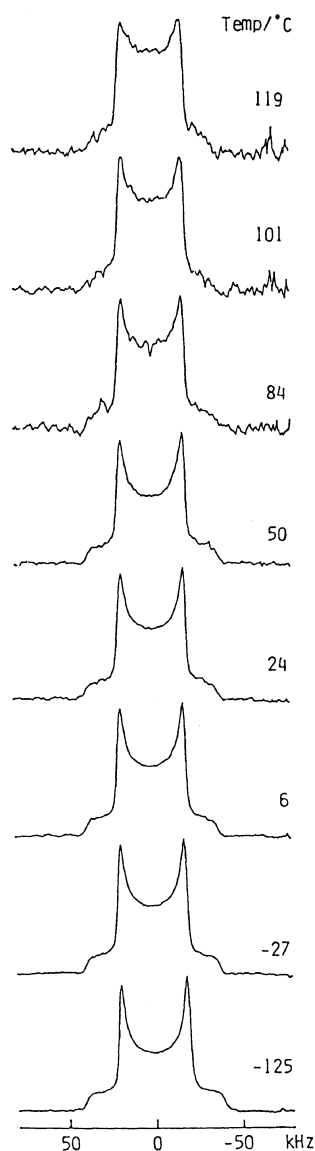


**Figure 1.** Temperature dependent $^2$H NMR spectra of PMLA-$d_3$. Lorentzian line broadening is 500 Hz. Note the intensity of spectra is normalized.

ponents of the spectra had a shorter $T_1$ than the perpendicular components (singularities) in the temperature range from $-126°$ to $20°$C. Such $T_1$ anisotropy is theoretically predicted for the 3-fold jump about the $C_3$ axis for the methyl group.[21] The temperature dependent $T_1$ for the singularities of the spectra is shown
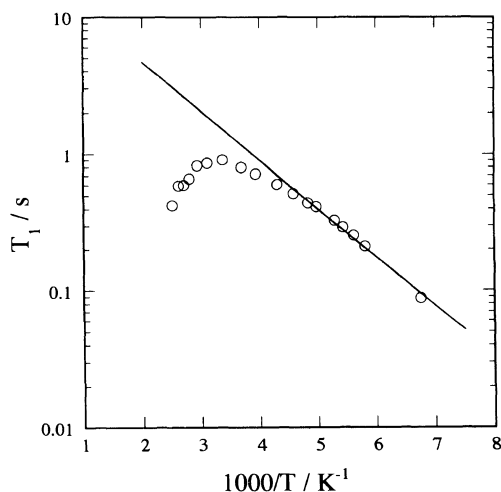
**Figure 2.** Temperature dependent $T_1$ of the singularities of the spectra for PMLA-$d_3$. The solid line is the calculated $T_1$ for the 3-fold jump with values in the text.



**Figure 3.** Calculated $^2$H NMR spectra visually fitted the experimental spectra at above 50°C in Figure 1. The rate of the 2-fold jump and temperature are (a) 1 kHz and 50°C, (b) 2 kHz and 84°C, (c) 4 kHz and 101°C, and (d) 6 kHz and 119°C.

in Figure 2. $T_1$ values increase linearly with temperature from $-125°$ to $-40°$C, showing the fast motion regime. It is possible to calculate the correlation time $(\tau_c)$ of the motion from the $T_1$ value using the 3-fold jump model described by Torchia and Szabo,[21] once the mechanism of motion has been determined. We assumed that the jump is a single activated process so that $\tau_c = \tau_0 \exp(E_a/RT)$. The Arrhenius plot yields an apparent activation energy of 6.8 kJ mol$^{-1}$ and preexponential factor of $1.5 \times 10^{-13}$ s. The correlation time is estimated to be $1.5 \times 10^{-11}$ s at $-95°$C. The activation energy obtained is the same as that for the methyl group of poly($\gamma$-methyl L-glutamate),[22] and is somewhat lower than that reported for the methyl groups in homopolypeptides.[23] These results imply that the methyl group bonded to oxygen atom experience a lower steric hindrance than a methyl group bonded to carbon atom, and support the view that the intermolecular contribution to the hindering barriers to the methyl group reorientation is relatively weak.

There are observable deviations from the linear Arrhenius plot behavior at above $-40°$C, together with slight decreases in
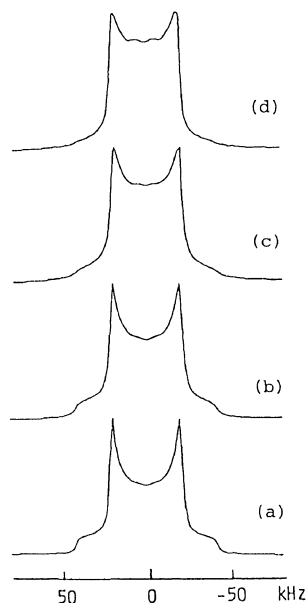
quadrupole splitting, indicating the onset of new and rapid motions in the side chain. As greatly affecting $T_1$ and showing only slight change of line shapes, they must be rapid, small-amplitude motions of the order of the Larmor frequency. It would not be possible to be of the exact nature of these new motions, without additional labeling experiments.

It was reported from the conformational energy calculation of PMLA that the $O_\delta$–$C_\varepsilon$ bond ($C_3$ axis) is trans and parallel to the $C_\beta$–$C_\gamma$ bond in the side chain.[24,25] Therefore, the rotational motions about the $C_\beta$–$C_\gamma$ little affect the line shapes of the methyl group, which was confirmed by the comparison between calculated and observed spectra (data not shown). Though rapid small-amplitude librations are accounted for the main chain motions of $\alpha$-helical poly($\gamma$-benzyl L-glutamate) in the $^2$H NMR spectra at the same temperature range studied,[26] such motions convinced to undergo in the main chain of helical PMLA would not

contribute to the line shapes of the methyl group. The line shapes observed at above 50°C suggest the presence of the slow motions about the $C_\alpha$–$C_\beta$ bond in the intermediate exchange described above.

In order to calculate line shapes, we assume the two-fold jump with equal population about the $C_\alpha$–$C_\beta$ bond and the effective quadrupole coupling constant of 50.7 kHz, in addition to the 3-fold jump of the methyl group. Furthermore, the rotational jump motions must be modeled by Gaussian distributions of jump angles.[27] The visually fitted calculating spectra are shown in Figure 3 with the averaged angle of 110° and the standard deviation of 5°. This angle obtained is in good agreement with the angle between the stable two positions of *trans* and *gauche'*, which was obtained from the conformational energy calculation of PMLA.[24,25] The gauche state is not allowed due to the severe steric conflict taking place between the backbone and the $C_\gamma$ carbonyl group. These results indicate that the reorientation about the $C_\alpha$–$C_\beta$ bond corresponds to a *trans-gauche'* isomerization. Since the jump rates of this motion are in the range of kHz, they do not contribute to the $T_1$ but the spectra in the higher temperatures show a characteristic of intermediate exchange.[4,15,16] The Arrhenius plot of the jump rate yields the apparent activation energy of about 28 kJ mol$^{-1}$. This is compatible with the value reported for the rotational jump in the side chain of L-methionine.[28] Additional direct support for this motion introduced in the line shape simulations will come from the deuteration of $\beta$ positions in this polymer, which is currently under investigation.

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