Proton NMR Relaxation Study of Molecular Motion in Poly(γ-*n*-octadecyl L-glutamate) and Poly(γ-oleyl L-glutamate)

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ABSTRACT: The proton NMR relaxations and proton line widths of poly(γ-*n*-octadecyl L-glutamate) and poly(γ-oleyl L-glutamate) in the solid state have been measured over a wide range of temperatures from −150 to +120 °C. From these experiments, it was found that a γ-relaxation exists in the former polymer and that γ- and β-relaxations exist in the latter. On the basis of these results, the molecular motions of the side chains and the α-helical main chain are discussed.

Introduction

Poly(γ-*n*-octadecyl L-glutamate) (POLG)[1] and poly(γ-oleyl L-glutamate) (POLLG)[2] form thermotropic liquid crystals above a certain temperature where the side chains of the polymers with α-helical backbone conformations assume a liquidlike structure. In order to clarify such behavior, ^{13}C CP/MAS (cross polarization/magic angle spinning) NMR experiments have been carried out as a function of temperature.[2-4] It has been found that in POLG the main chain assumes a right-handed α-helical conformation over the temperature range from 27 to 100 °C. The side chains assume an all-trans zigzag conformation in the crystalline state at room temperature and are in a mobile state above the melting temperature of the side-chain crystals, while the main chain is undergoing molecular motion at a frequency of about 60 kHz at 40 °C (in the liquid-crystalline state). On the other hand, POLLG may be in a liquid-crystalline state even at room temperature, since the long flexible side chains cannot form a crystalline phase even on cooling to −40 °C due to the effect on unsaturated double bonds. In order to understand the nature of these thermotropic behaviors exhibited by such polypeptides, it is important to collect detailed information about their dynamic behavior over a wide range of temperatures.

The purpose of this work is to investigate the dynamics of POLG and POLLG through the observation of proton spin–lattice relaxation times (T_1) and $T_{1\rho}$ and spin–spin relaxation times (T_2) over a wide range of temperatures. In addition, the effect of saturated and unsaturated side chains is discussed through the comparison of these results.

Experimental Section

Materials. POLG and POLLG were synthesized by ester-exchange reactions between poly(γ-methyl L-glutamate) (M_v = 100 000) and the corresponding alcohols. Each reaction was carried out in 1,2-dichloroethane with *p*-toluenesulfonic acid as a catalyst at 60 °C.[5] The complete replacement of methyl groups by octadecyl or oleyl groups was confirmed by their ^{1}H NMR spectra recorded in solution.

Measurements. Proton relaxation times T_1, T_2, and $T_{1\rho}$ of the sample were measured with a Bruker CXP-90 NMR spectrometer operating at 90 MHz as a function of temperature using the inversion–recovery (180°–τ–90°) method, the solid echo method, and the spin-locking method, respectively. The locking field, H_1, was 1.0 mT. ^{1}H NMR spectra were obtained by Fourier transforming the solid echo signal. Pieces of the samples were packed into NMR tubes, followed by degassing and sealing off procedures.

Analysis of Relaxation Times. Relaxation times T_1 and $T_{1\rho}$ are long for polymers in the solid state, where the molecular motion is frozen or considerably restrained. Elevation of temperature leads to an increase in molecular motion of polymers in the solid state (the decrease of the correlation time, τ_c), and so T_1 and $T_{1\rho}$ decrease. A minimum in T_1 is reached at $\omega_0\tau_c = 2\pi\nu_c\tau_c = 1$, where ω_0 and ν_c are the resonance frequencies in radians per seconds and in hertz, respectively, and T_1 again increases.[6] From the T_1 minimum, we can obtain the correlation time τ_c for molecular motion at megahertz frequencies. On the other hand, $T_{1\rho}$ shows T_1-like behavior against temperature, but a minimum in $T_{1\rho}$ is reached at $\omega_1\tau_c = 1$, where $\omega_1/2\pi = \gamma H_1/2\pi = 42.6$ kHz.[7] From the $T_{1\rho}$ minimum, we can obtain the correlation time, τ_c, for molecular motion at kilohertz frequencies. It is also useful to use T_2 for the analysis of the slow motion of polymers. The solid echo signal consisting of two components with shorter T_2 and longer T_2 can sometimes be resolved into the corresponding components by a Gaussian function, $\exp[(-t/T_2)^2/2]$ and a Lorentzian function, $\exp(-t/T_2)$, respectively.

Results and Discussion

POLG. ^{1}H NMR spectra of POLG were measured over the range of temperatures from −80 to +100 °C. Some typical ^{1}H NMR spectra of POLG at various temperatures are given in Figure 1. As seen from the ^{1}H NMR spectra at temperatures from 20 to 50 °C, the ^{1}H signal consists of two components. One is a broad component, which comes from protons in immobile regions, and the other is a narrow component, which comes from protons in mobile regions. From an analysis of the signal, it is found that the line shape for the broad component is Gaussian and that for the narrow component is Lorentzian. In order to obtain the fraction of each component, the solid echo decay was analyzed as described above. The fraction of a longer T_2 component (mobile fraction) so determined is shown as a function of temperature in Figure 2. The mobile fraction increases in the temperature range 40–60 °C. This may be attributable to the melting of side-chain crystallites formed in this kind of polypeptide with long alkyl side chains. In a previous paper,[1] the DSC measurement showed that the melting transition occurs at 62 °C, which agrees with the temperature observed here. Above the melting temperature of the side-chain crystallites, the mobile fraction becomes constant at 0.91, with an immobile component of 0.09. These fractions correspond to the main-chain protons (NH and C_αH) and the side-chain CH_2 protons, respectively. Such a separation of T_2 components characterizes the molecular motions in this kind of polypeptide.

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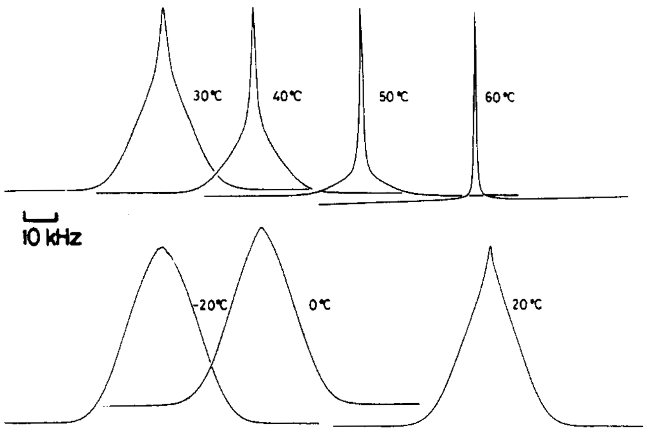

Figure 1. ^{1}H NMR spectra of poly(γ-*n*-octadecyl L-glutamate) at various temperatures.

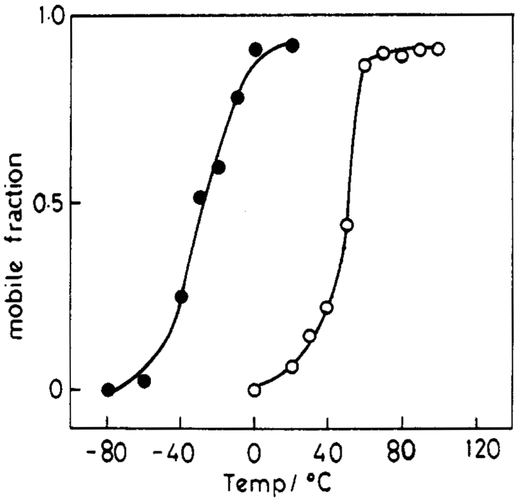

Figure 2. Variation of mobile fraction of poly(γ-*n*-octadecyl L-glutamate) (○) and poly(γ-oleyl L-glutamate) (●) with temperature as determined by ^{1}H NMR spectra.

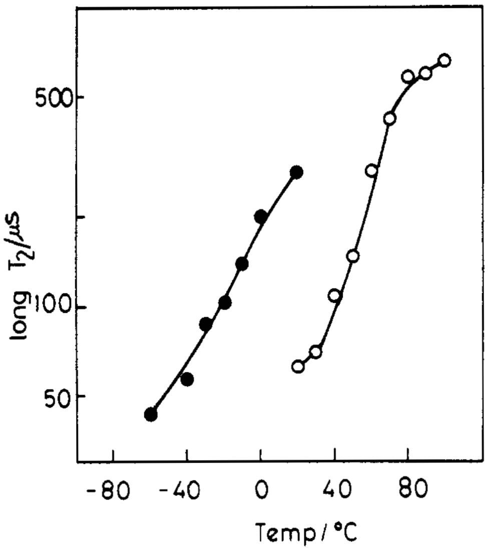

Figure 3. Temperature dependence of T_2 for the mobile components of poly(γ-*n*-octadecyl L-glutamate) (○) and poly(γ-oleyl L-glutamate) (●).

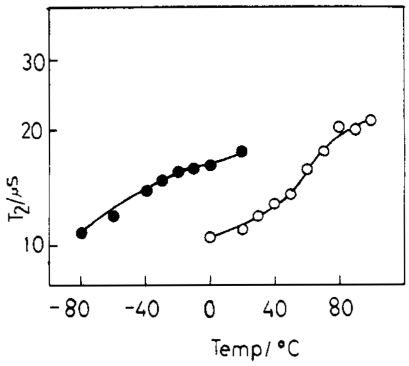

Figure 4. Temperature dependence of T_2 for the immobile components of poly(γ-*n*-octadecyl L-glutamate) (○) and poly(γ-oleyl L-glutamate) (●).

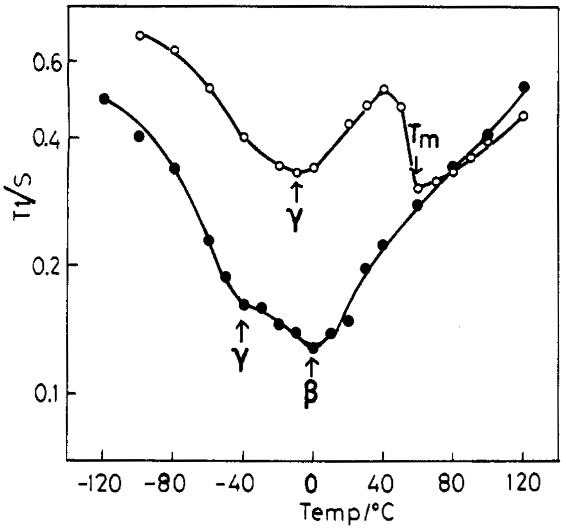

Figure 5. Temperature dependence of T_1 for poly(γ-*n*-octadecyl L-glutamate) (○) and poly(γ-oleyl L-glutamate) (●): β, β-relaxation; γ, γ-relaxation; T_m, melting point of the side-chain crystallite.

The melting transition of side-chain crystallites is also reflected in the T_2 values. The T_2 values for the mobile component are shown as a function of temperature in Figure 3. This shows that the T_2 value undergoes a transition at a temperature between 40 and 70 °C. On the other hand, the T_2 value for the shorter T_2 component increases slowly and monotonously as the temperature is increased, as shown in Figure 4. This means that the mobility of the main chain increases continuously with no transition, in contrast with the behavior of the side chains.

In order to describe the details of molecular motion, T_1 and $T_{1\rho}$ values are plotted against temperature as shown in Figures 5 and 6, respectively. The magnetization recovery and decay are a single exponential for each series of experimental data. According to the BPP theory,[6] T_1 passes through a minimum and increases again when the correlation time, τ_c, for molecular motion increases further. $T_{1\rho}$ behaves similarly to T_2 and is more sensitive to lower frequency motions.[7] As can be seen from Figure 5, T_1 of POLG decreases from 700 to 350 ms as the temperature is increased from −100 to −10 °C. This means that the molecular motion is in the slow-motion region; i.e., $\omega\tau_0 \gg 1$. Above −10 °C, T_1 increases from 350 to 550 ms as the temperature is increased from −10 to +45 °C. This means that the molecular motion is nearly in the extreme narrowing region ($\omega\tau_c \ll 1$). (The relaxation arises from the side-chain motion, which corresponds to the γ-relaxation observed in viscoelastic measurements.[1] This can be justified from the Arrhenius plot as shown in Figure 7.) However, T_1 decreases from 550 to 327 ms as the temperature is increased from 45 to 60 °C and again increases from 327 to 403 ms as the temperature is further increased from 60 to 120 °C. The minimum at lower temperature depends on the observing frequency, but that at higher temperature does not (see $T_{1\rho}$ results). As shown

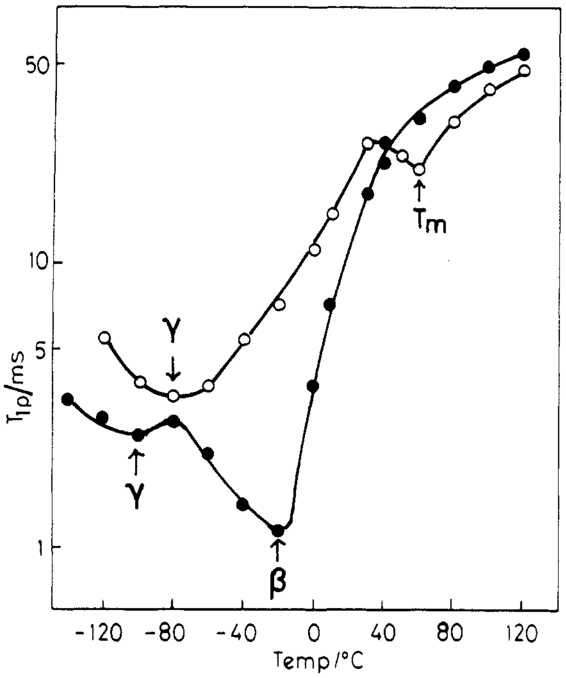

Figure 6. Temperature dependence of $T_{1\rho}$ for poly(γ-*n*-octadecyl L-glutamate) (○) and poly(γ-oleyl L-glutamate) (●): β, β-relaxation; γ, γ-relaxation; T_m, melting point of the side-chain crystallite.

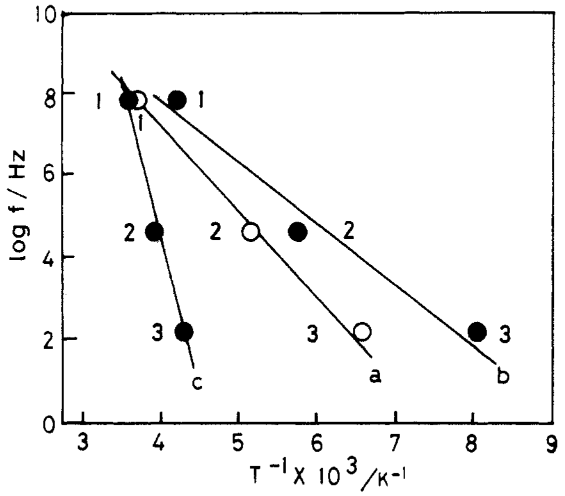

Figure 7. Arrhenius plots of log *f* in poly(γ-*n*-octadecyl L-glutamate) (a) and poly(γ-oleyl L-glutamate) (b and c) against the inverse of the absolute temperature. *f* is the frequency in hertz. (a and b) From γ-relaxation. (c) From β-relaxation. 1 and 2 were obtained from T_1 and $T_{1\rho}$, and 3 was obtained from the viscoelastic data (ref 1).

in Figure 5, the first minimum comes from relaxation and the second one comes from the first-order melting transition.

We now discuss the $T_{1\rho}$ results. The $T_{1\rho}$ values are plotted against temperature in Figure 6. Two distinct minima are observed. $T_{1\rho}$ decreases from 6.0 to 3.0 ms as the temperature is increased from −120 to −80 °C and increases from 3.0 and 29 ms through the first minimum as the temperature is further increased. Again, the $T_{1\rho}$ value decreases from 29 to 21 ms as the temperature is increased from 30 to 60 °C and increases from 21 to 41 ms through the second minimum as the temperature is further increased from 60 to 120 °C.

Next, we are concerned with the molecular motion related to the γ-relaxation. It can be said that below −80 °C the γ-relaxation comes from the molecular motion corresponding to the rotation of methyl groups in the side chains at a frequency below ca. 40 kHz as seen from the $T_{1\rho}$ minimum. The activation energy, ΔE, for the γ-relaxation can be determined by using $\tau_c = \tau_0 e^{-\Delta E/RT}$, where τ_0 is the prefactor, R is the gas constant, and T is the absolute temperature. (An accurate correlation time, τ_c, can be estimated using $\tau_c = 1/2\pi\nu_c$.) Therefore, the activation energy, ΔE, was determined from the plots of log *f* (the frequency in hertz) against T^{-1} as shown in Figure 7, where the values of *f* for the T_1 and $T_{1\rho}$ minima are 90 MHz and 42.6 kHz, respectively. The activation energy of POLG is 10 kcal/mol. This is a reasonable value for the γ-relaxation and agrees with that (11 kcal/mol) obtained from the mechanical relaxation by viscoelastic measurement as reported previously.[1]

POLLG. The ^{1}H NMR spectra for POLLG were measured as a function of temperature (not shown). It is found that the ^{1}H NMR spectra have two components over a wide range of temperatures. The fraction of the longer T_2 component (mobile fraction) is plotted against temperature in Figure 2. The mobile fraction increases monotonously as the temperature is increased from −80 to 0 °C, and it becomes constant, being 0.9, at temperatures above 0 °C. The fraction of 0.1 for the shorter T_2 component comes from the main-chain protons, NH and $C_\alpha H$, and the side-chain proton $C_\beta H_2$.

The T_2 value for the mobile component is shown as a function of temperature in Figure 3. The T_2 value increases transitionally at temperatures between −40 and +20 °C. Its transitional change is not sharp. However, the T_2 value for the shorter T_2 component increases slowly and monotonously as the temperature is increased (Figure 4). This means that the mobility of the main chain increases continuously with no transition.

The T_1 values of POLLG are plotted against temperature in the temperature range from −120 to +120 °C in Figure 5. T_1 decreases from 504 to 138 ms as the temperature is increased from −120 to 0 °C. The value of T_1 increases from 138 to 561 ms as the temperature is further increased from 0 to 120 °C. Only one minimum is observed in the temperature range from −120 to +120 °C. However, if we look at the relaxation curve carefully, it seems that there may be two overlapping relaxations in the vicinity of −40 and 0 °C. The separation of the two minima is unclear. In order to clarify this problem, the values of $T_{1\rho}$ are plotted against temperature as shown in Figure 6. $T_{1\rho}$ decreases from 3.5 to 2.5 ms as the temperature is increased from −120 to −100 °C and increases from 2.5 to 2.9 ms through the first minimum as the temperature is further increased from −100 to −80 °C. The $T_{1\rho}$ value decreases from 2.9 to 1.2 ms as the temperature is increased from −80 to −20 °C and increases from 1.2 to 49 ms through the second minimum as the temperature is increased from −20 to +120 °C. Two distinct minima clearly appear. The first minimum at about −100 °C is the γ-relaxation, and the second one at about 30 °C is the β-relaxation. This explanation is similar to the case of POLG.

When the experimental data on the two minima in the relaxation curve are used, the activation energies for both relaxations were determined by plotting log *f* versus the inverse of absolute temperature (Figure 7b). The activation energy for the γ-relaxation so determined was about 7 kcal/mol, which is smaller than that of POLG. This means that the oleyl side chains are more flexible than the *n*-octadecyl side chains. This is consistent with the conclusion that POLLG is in a liquid-crystalline state over a wide range of temperature.

We are also concerned with the activation energy for the β-relaxation (Figure 7c). The β-relaxation arises from the overall motion of the side chains. The activation energy found for the β-relaxation was about 37 kcal/mol. This value is larger than that for the γ-relaxation but is reasonable compared with the β-relaxations of other polymers.[8]

From the above results for POLG and POLLG, it is concluded that the existence of an unsaturated double bond in the oleyl groups of the latter polymer leads to substantially different molecular motion compared with the former polymer with n-alkyl groups having the same number of carbons and produces notable differences in their physical properties.

References and Notes

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