SHORT COMMUNICATIONS

Deuterium NMR Studies of Benzene-d_6 in Poly(L-leucine) Gel

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Solutions of rigid-rod polymers have been found to undergo gelation. In these systems, extreme chain rigidity is attained through chemical structure, intramolecular interactions, and/or interactions between a polymer and a solvent. Flory described that the lyotropic liquid-crystal state may form when the rigid-rod polymers dissolve in good solvents above the critical concentration.[1,2] However, the behavior of rigid-rod polymers in poor solvents would be different. Most of the work has been reported for an α-helical poly(γ-benzyl L-glutamate) (PBLG).[3-5] The preferential reorientations of methylenechloride adsorbed was observed on PBLG solid film, due to the interactions between the rod-like molecule and the solvent.[6]

Another rigid-rod homopolypeptide that can form gels is poly(L-leucine) (PLL) in benzene.[7] The gel-sol transition in the benzene solution of PLL was reported to occur at around 50°C by various methods.[7-12] In this work we carried out deuterium NMR measurements to investigate the dynamics of benzene in PLL solution and to present further evidences of the transition. The concentration and temperature dependence of the relaxation times provides information about the solution structure and the benzene dynamics.

The deuterium relaxation has the advantage compared with protons that there is no interfering signal from the polymer. The deuteron is a quadrupolar nucleus so that the intrinsic relaxation time is governed by intramolecular rotational modulation of the electric quadrupolar interaction. Since there is no exchangeable deuterons in the present system, the chemical exchange process between the solvent and the exchangeable nucleus in polymer, which is important relaxation process in the aqueous carbohydrate system,[13-15] is negligible.

EXPERIMENTAL

Poly(L-leucine) (PLL) was synthesized by the N-carboxyanhydride (NCA) method using triethylamine as the initiator in benzene. Sample was prepared by mixing the polymer and the mixture of perdeuterated benzene (20% v/v) and benzene, and the sample tube was sealed off. Then, the sample was heated up to temperature around 75°C. Complete dissolution of the polymer was taken to be the point at which uniform transparency was achieved. The solutions were allowed to cool to room temperature, during which process the gel formed. Deuterium NMR measurements
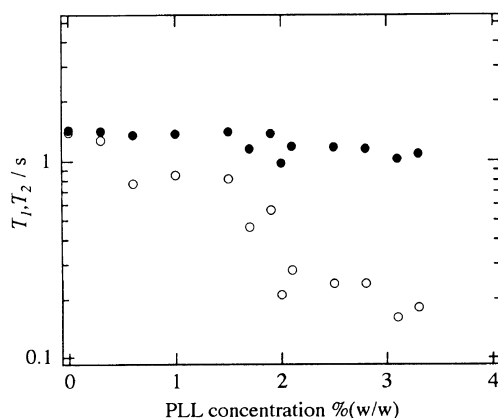
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were carried out on a JEOL FX90Q spectrometer at 13.7 MHz. Relaxation times, T_1 and T_2, were determined by the inversion-recovery and CPMG pulse sequences, respectively. Single exponential relaxation was observed in all cases.

RESULTS AND DISCUSSION

Figure 1 shows the PLL concentration dependence of the relaxation times of benzene deuterons at 25°C in the gel. The relaxation times decrease in the presence of PLL and T_2 becomes much shorter than T_1, while in the absence of PLL T_1 is equal to T_2. These results indicate the presence of the slow motional states and/or the exchange process of benzene molecule. Remarkable change in T_2 is observed around the concentration of 2% w/w, suggesting the different motional states of the solvent in the gel dependent on the PLL concentration. Similar results were obtained for benzene protons.

We can make reasonable assumptions that there are two types of benzene molecules in the system, that is, benzene bound to PLL and free benzene, and that benzene undergoes the chemical exchange between the bound and free states with an exchange rate faster than a relaxation rate in the free state. Under the condition of the chemical exchange, the contribution of PLL to the spin–spin relaxation time of benzene T_{2p} is expressed by[16]

$$\frac{1}{T_{2p}} = \frac{1}{T_2} - \frac{1}{T_{20}} = \frac{f}{T_{2b} + \tau_m} \qquad (1)$$

where T_2 and T_{20} are the spin-spin relaxation times observed in the presence and absence of PLL, respectively, T_{2b} is the spin-spin relaxation time of the bound benzene, τ_m is the life time of the bound benzene, and f is the fraction of the bound benzene. T_{2p} is determined by T_{2b} and τ_m, which have different temperature dependence. T_{2b} can be evaluated by assuming an isotropic motion with the correlation time τ_c so that[17]

$$\frac{1}{T_{2b}} = \frac{\pi^2}{20} \chi^2 \left(1 + \frac{\eta^2}{3} \right)$$
$$\times \left(9\tau_c + \frac{15\tau_c}{1 + \omega^2 \tau_c^2} + \frac{6\tau_c}{1 + 4\omega^2 \tau_c^2} \right) \qquad (2)$$

where χ is the quadrupole coupling constant, η the asymmetry parameter, ω the Larmor frequency, and τ_c is given by the Arrhenius-type temperature dependence. The values of χ and η for benzene-d_6 are 183 kHz and 0.04, respectively.[18] Thus, T_{2b} is an increasing function of temperature in the experimental conditions used. On the other hand, τ_m decreases with increasing temperature, assuming the Erying relationship for the first-order reaction of the chemical exchange.

The f value can be estimated from the molecular dimensions of PLL and benzene. PLL forms an α-helical rod-like molecule with the length of 75 nm and the radius of about 0.8 nm which are evaluated from the molecular weight ($MW = 56000$) and the molecular model of PLL. And PLL is close-packedly covered with benzene molecules of van der Waals radius of 0.38 nm. The concentration of 1% w/w corresponds to $f = 11 \times 10^{-3}$ under the conditions. Here, the value of f is assumed to be constant in the solution conditions used.

Figure 2 shows the temperature dependence

Figure 1. PLL concentration dependence of T_1 (●) and T_2 (○) for benzene-d_6 at 25°C.
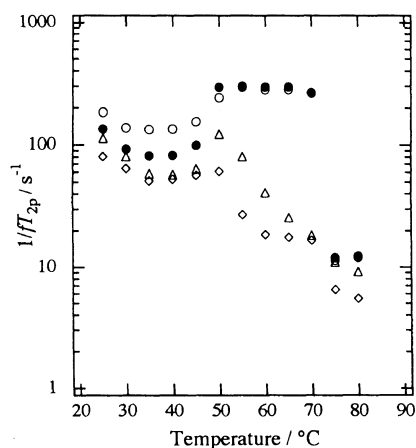
Figure 2. Temperature dependence of $1/fT_{2p}$: $\triangle$, 1.0% w/w; $\diamondsuit$, 1.9% w/w; $\bigcirc$, 2.0% w/w; $\bullet$, 3.3% w/w.

of $1/fT_{2p}$. With increasing temperature, $1/fT_{2p}$ starts to increase from 35°C, reaches a maximum at about 50°C, and decreases for the lower concentration samples less than 2% w/w. Similar temperature dependence of $1/fT_{2p}$ was reported for the gel systems.[13,19] The plateau region of $1/fT_{2p}$ is observed in the temperature range of 50 to 70°C for the higher concentrations more than 2% w/w, and $1/fT_{2p}$ decreases abruptly above 70°C. The temperature of about 50°C is in good agreement with the gel–sol transition temperature determined by the falling ball method[20] and other methods.[7-12] The temperature width of the gel–sol transition is almost the same within a several degrees irrespective of the PLL concentration. These results indicate that dynamics of benzene molecules remarkably changes in the gel–sol transition temperature. The reorientations of PLL molecules is as well allowed after the transition.[8,10]

Since τ_m contributes to $1/fT_{2p}$ in the gel state between 35 to 50°C, τ_m can be calculated to be 17 ms at 35°C for the 1% w/w sample. With increasing temperature, τ_m decreases to 8 ms at 50°C and will decrease further. The activation energy of removal of benzene from PLL in the gel state can be estimated to be approximately 23 kJ mol^{-1} from the temperature dependence

of τ_m. This is comparable to the values obtained for agar and κ-carraggenan gels,[13,19] although a different solvent (water) is used in these systems.

Since $1/fT_{2p}$ shows a maximum at about 50°C and decreases with increasing temperature in sol state for the lower concentrations, T_{2b} contributes to $1/fT_{2p}$ and, therefore, τ_c is calculated to be about 40 ns at 50°C from eq 2. On the other hand, τ_c of free benzene could be calculated to be 1.2 ps at 50°C. These results indicate that the bound benzenes are remarkably restricted in the sol state. The Arrhenius plot of τ_c is found to be the activation energy of about 100 kJ mol^{-1}. This value is much larger than 7.0 kJ mol^{-1} in the free benzene obtained. The α-helical PLL molecule in benzene could be considered as hydrodynamically equivalent to a prolate ellipsoid. The three rotational correlation times characteristic of this model can be calculated to be 180 ns, 4 ns, and 1 ns at 50°C, according to Woessner.[21] Since these values are quantitatively close to τ_c for the bound benzene, τ_c and the activation energy obtained would reflect the dynamics of the PLL molecule.

Electron spin resonance and small-angle X-ray scattering studies on spin-labeled PLL in benzene indicated the presence of the ordered molecular aggregates in the temperature of 50 to 70°C.[8,11,12] Differential scanning calorimetric (DSC) studies showed the exothermic peak just after the endothermic peak that shows the melting of the gel, suggesting the formation of the ordered state.[20,22] The anisotropic phase was observed in the same temperature region by optical polarized microscopy.[20] The characteristic profile of $1/fT_{2p}$ showing a plateau seems not to be observed for any gel systems.

The single resonance without the quadrupole splitting was observed, even after leaving the sample in the higher magnetic field (6.3 T) for more than a day. This is in contrast to the liquid-crystal or the gel in α-helical poly(γ-benzyl L-glutamate),[23,24] in which was observed the quadrupole splitting of the deu-

terated solvent resonance. The isotropic resonance is attributed to the fact that the PLL concentration used is theoretically not above the critical concentration for liquid-crystal formation.[1,2] Thus, one would expect the plateau of $1/fT_{2p}$ to exhibit the ordered structure. Applying the eq 1 and 2 to the plateau region, τ_c would be calculated to be about 120 ns between 50 to 70°C. This value is one order larger than that in the sol, suggesting the motional restriction of benzene molecules. Decreasing of $1/fT_{2p}$ above 70°C corresponds to the melting from this structure to the sol, confirmed by the DSC measurements.[20,22]

Since Figure 1 shows the definite difference in T_2 values at 25°C, observed T_2 in the gel may represent the potential criterion of the distinction between the ordered and the sol states after melting the gel. Figure 2 shows slight decrease in $1/fT_{2p}$ from 25 to 35°C. This suggests that in the gel state and in the lower temperatures T_{2b} may contribute to T_{2p}, rather than τ_m. T_{2b} in the sol state have discussed above. In this case we might assess dynamics of the bound benzene on PLL in the gel.

In conclusion, gel–sol and gel–ordered state–sol transitions are detected with deuterium spin–spin relaxation of benzene in the PLL solution. Dynamics of solvent molecules reflects the state of the solution.

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