Self-Diffusion Behavior of Solvents in Polypeptide Gel As Studied by Pulsed-Gradient Spin-Echo ^{1}H NMR Spectroscopy

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Introduction

In high-resolution solid-state ^{13}C NMR experiments on cross-linked poly(γ-methyl L-glutamate) (PMLG) gel, we have elucidated that the conformation of cross-linked PMLG with a mixture of chloroform ($CHCl_3$) and trifluoroacetic acid (TFA) largely depends on the solvent composition.[1] The increase of volume fraction of TFA (f_{TFA}) in a mixture of $CHCl_3$ and TFA induces the α-helix−random coil transition of the main chain of cross-linked PMLG. $CHCl_3$ and TFA are sometimes so-called "helix" and "coil" solvents, respectively. It is significant for gel science in polypeptide gel systems to elucidate how the translational motion of these solvents is affected when the main chain of PMLG changes from the α-helix form to the random coil form. In such system, the diffusion of solvent molecules contained in the polypeptide gel system strongly depends on the dynamics of polypeptide chains. Recently, it has been demonstrated that the pulsed-gradient spin-echo (PGSE) ^{1}H NMR method gives very useful information about the diffusion process of a polymer gel system.[2−5] Further, the microscopic molecular motion of solvent[4−8] and probe polymer[3,4] in polymer network has been studied by the observation of spin−spin relaxation time (T_2) and spin−lattice relaxation time (T_1).

From such a background, we aim to elucidate the dynamics of solvents in cross-linked PMLG gel with a mixture of $CHCl_3$ and TFA on going from the α-helix form to the random coil form through the diffusion coefficients determined by PGSE ^{1}H NMR.

Experimental Section

Materials and Gel Preparation. Poly(γ-methyl L-glutamate) (PMLG) was kindly supplied by Ajinomoto Co. Ltd., of which the molecular weight is 130 000. The PMLG was cross-linked by diaminododecane (20 mol %/monomer unit) as a cross-link reagent through the ester−amide exchange reaction to prepare the gel. The cross-linking reaction was performed at 343 K for 7 days in 1,4-dioxane at the polymer concentration of 15% (w/v). The gel obtained was swollen in chloroform ($CHCl_3$), trifluoroacetic acid (TFA), and a mixture of $CHCl_3$ and TFA with various volume fractions.

Measurements. The self-diffusion coefficient measurements on $CHCl_3$ and TFA in a PMLG gel were carried out by means of a JEOL GSX-270 NMR spectrometer operating at 270.1 MHz for ^{1}H with a home-made pulse gradient generator using a standard PGSE pulse sequence from 298 to 318 K. Both of the field gradient strengths, 5.9 and 12.8 T m^{-1}, were used. Spectral width and data points were 4.0 kHz and 4096, respectively.

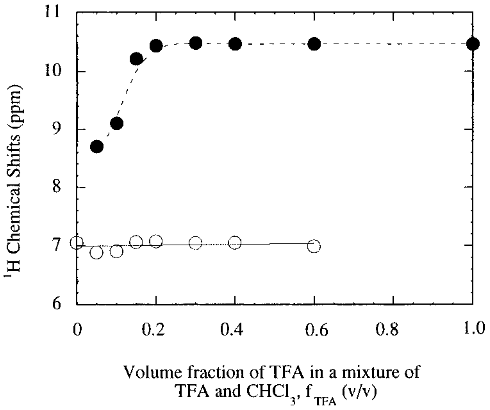

Figure 1. Plots of ^{1}H chemical shifts for TFA and $CHCl_3$ in the PMLG gel system against the TFA content in a mixture of TFA and $CHCl_3$: TFA (●) and $CHCl_3$ (○).

The relationship between echo signal intensity and pulse field gradient parameters is given by

$$A(\delta)/A(0) = \exp[-\gamma^2 G^2 D\delta^2(\Delta - \delta/3)] \qquad (1)$$

where $A(\delta)$ and $A(0)$ are echo signal intensities at $t = 2\tau$ with and without the magnetic field gradient pulse being the length δ, respectively. τ is the pulse interval, γ the gyromagnetic ratio of the proton ($\gamma = 2.675 \times 10^{11}$ rad G^{-1} s^{-1}), G the field gradient strength, D the self-diffusion coefficient, and Δ the gradient pulse interval. The echo signal intensity was measured as a function of δ. The plot of $\ln[A(\delta)/A(0)]$ against $\gamma^2G^2\delta^2(\Delta - \delta/3)$ gives a straight line with a slope of $-D$. Therefore, the D value can be determined from its slope. The τ, Δ, and δ values employed in these experiments were 30 ms, 4−10 ms (for gel) and 30 ms (for solvents), and 0.001−0.40 ms (for gel) and 0.001−0.12 ms (for solvents), respectively.

Results and Discussion

Spin-echo ^{1}H NMR spectra of a PMLG gel containing a mixture of $CHCl_3$ and TFA with $f_{TFA} = 0.10$ were measured as a function of magnetic field gradient pulse lengths (δ). In the spectra (not shown here), the two intense peaks at 6.9 and 9.1 ppm were straightforwardly assigned to the $CHCl_3$ and TFA protons, respectively. The ^{1}H chemical shift of $CHCl_3$ almost does not change with an increase in TFA content, as shown in Figure 1. On the other hand, The ^{1}H chemical shift of TFA is observed at 8.7−10.5 ppm with an increase in TFA content.

Figure 2 shows the degree of swelling (Q) of PMLG gel in a mixture of TFA/$CHCl_3$ against the TFA content (f_{TFA}). A PMLG gel in $CHCl_3$ is transparent. The degree of swelling decreases with increasing TFA content from $f_{TFA} = 0.05$ to 0.30, while the gel becomes turbid. In the TFA content from $f_{TFA} = 0.30$ to 1.0, the degree of swelling of the PMLG gel increases with increasing TFA content, meanwhile the gel becomes

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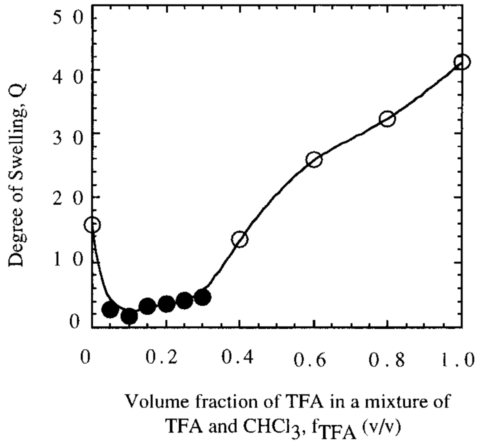

Figure 2. Dependence of the degree of swelling (Q) of the PMLG gel on the TFA content in a mixture of $CHCl_3$ and TFA: (○) the gel is transparent; (●) the gel is turbid.

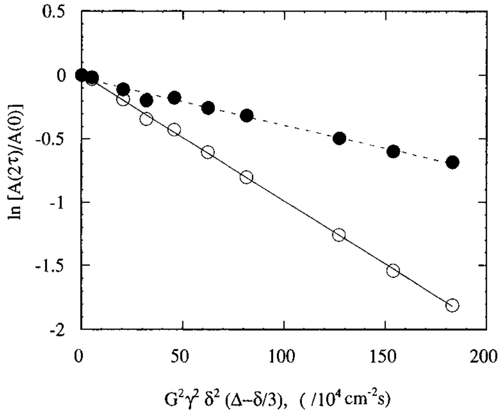

Figure 3. Diffusional spin-echo attenuation of TFA (●) and $CHCl_3$ (○) in PMLG gel with $f_{TFA} = 0.10$ by varying the field gradient pulse duration (δ). From the slopes of the solid and dashed straight line, D_{TFA} and D_{CHCl_3} were obtained to be 0.37 × 10^{-5} and 1.00 × 10^{-5} $cm^2\ s^{-1}$, respectively.

transparent again. This shows that the main chain of the PMLG gel underwent a conformation transition from the α-helix form to the random coil form. The mixture solvent with $f_{TFA} = 0.05-0.30$ acts as a poor solvent to the random coil form; the inhomogeneous aggregation occurs in the gel system. The mixture solvent with higher TFA content as $f_{TFA} = 0.30-1.0$ acts as a good solvent to the random coil form.

The diffusion coefficients of TFA and $CHCl_3$ contained in a PMLG gel were determined by the PGSE ^1H NMR method at 303 K by varying the TFA content contained. The decays of TFA and $CHCl_3$ lie on a straight line, as shown in Figure 3. This means that the individual diffusants of TFA and $CHCl_3$ in PMLG gel have a single diffusion component during the observation time. From the slope of the intensity decay, the diffusion coefficients for TFA (D_{TFA}) and $CHCl_3$ (D_{CHCl_3}) can be determined by eq 1 to be 0.37 × 10^{-5} and 1.00 × 10^{-5} $cm^2\ s^{-1}$, respectively. The diffusion coefficient of TFA is smaller than that of $CHCl_3$. This means that the translational motion of TFA is more restrained by the PMLG network compared with $CHCl_3$. The diffusion coefficients of TFA and $CHCl_3$ in PMLG gel were plotted against the TFA content in a mixture of TFA/$CHCl_3$, as shown in Figure 4. As seen from this figure, D_{CHCl_3} largely decreases in the f_{TFA} range from 0 to 0.05. This tendency is very similar to that of the degree of swelling in Figure 2. This shows that the conformation of PMLG gel changed from the α-helix to the random coil with increasing TFA content. The transition from the α-helix form to the random coil form leads to the shrinkage of the PMLG gel due to the decrease of the network size and leads to the decrease of diffusion coefficients of $CHCl_3$. In the f_{TFA} range from 0.05 to 0.20, D_{TFA} and D_{CHCl_3} increase, although the degree of swelling is almost constant. It can be said that in the f_{TFA} range the helix–coil transition leads to such diffusional behaviors. After the helix–coil transition, the molecular motion of TFA and $CHCl_3$ is increased with an increase in f_{TFA}.

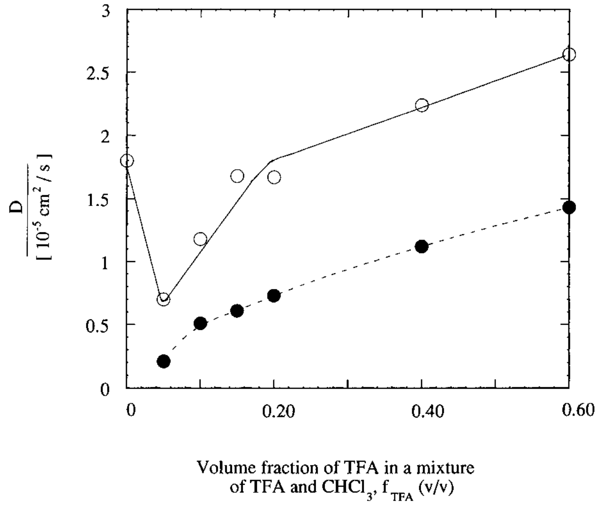

Figure 4. Plot of the diffusion coefficients for TFA (●) and $CHCl_3$ (○) in PMLG gel against the TFA content in a mixture of TFA and $CHCl_3$.

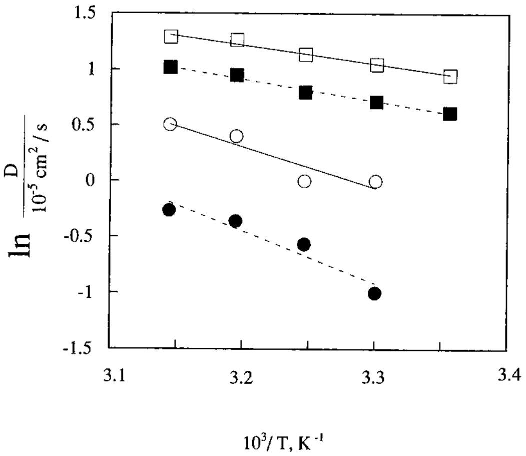

Figure 5. Temperature dependence of self-diffusion coefficients of TFA and $CHCl_3$ at $f_{TFA} = 0.10$ in the PMLG gel system, (●) for TFA and (○) for $CHCl_3$, and in a mixture solvent of $CHCl_3$ and TFA, (■) for TFA and (□) for $CHCl_3$.

The plots of ln D for TFA and $CHCl_3$ in PMLG gel are shown against the inverse of absolute temperature ($1/T$) in Figure 5. The diffusion coefficients can be expressed against temperature as an Arrhenius-like equation

$$D = (a_0^{\,2}/\tau_0)\exp(-E/kT) \qquad (2)$$

where a_0 is the interval of the diffusant, τ_0 the time of diffusing, k the Boltzmann constant, and E an activation energy for diffusion. From the slope for such plots, the activation energies for TFA and $CHCl_3$ were obtained to be 38.7 and 30.5 kJ/mol, respectively. On the other hand, the activation energies for pure TFA and $CHCl_3$

were obtained to be 16.2 and 13.5 kJ/mol, respectively. From these results in the gel system, it can be said that intermolecular interactions between solvent and polypeptide network are strong enough to restrain the translational motion.

References and Notes

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