Communications to the Editor

NMR Imaging of Diffusion of Small Organic Molecules in Silk Fibroin Gel

Introduction. NMR imaging is a modification of NMR spectroscopic techniques that uses linear magnetic field gradients to spatially encode the spins according to frequency and phase and has been widely and successfully employed in medical science.[1] However, application in polymer science has been slow to appear.[2-9] This is due in part to the fact that commercial NMR imaging apparatus suitable for such studies has become available only recently.

Although much interest surrounds small molecules in polymers, little information is available on the physical distribution of these molecules within the polymer or on the mechanism by which absorption or desorption occurs.[6,7] NMR imaging has the advantage that it does not disturb the diffusion process unlike most other techniques, which require stopping the diffusion process and destroying the sample.[3-7] Therefore, we have applied this imaging technique to the study of the diffusion process of small organic molecules into a silk fibroin gel swollen in water.

Silk fibroin has been found to be an excellent enzyme-immobilization material.[10] Recently, we reported the immobilization of glucose oxidase in a *Bombyx mori* silk fibroin membrane and the application of this membrane to a biosensor system capable of easily and continuously monitoring the concentration of glucose.[11,12] The immobilizations of lipase in silk fibroin,[13] of invertase in silk powder,[14] and of alkaline phosphatase in silk fiber[15] have also been tried. In order to develop improved enzyme-immobilized materials with silk fibroin, it is necessary to characterize in detail the states or diffusion of substrate in silk fibroin gel as well as the immobilized state of the enzyme.[16]

In our previous papers, the states of substrate and enzyme immobilized in silk fibroin membrane in the swollen state were studied with spin-label ESR,[14,16,17] high-resolution multinuclei NMR,[18-20] and ^{13}C cross polarization/magic angle spinning NMR.[21] In this paper, we will describe an NMR imaging study of the diffusion of a paramagnetic substance into silk fibroin gel. In particular, the diffusion coefficient, which provides important information concerning the extent of translation of small molecules in their environment, was determined.

Experimental Section. The ca. 8 w/v % aqueous solution of *Bombyx mori* silk fibroin was obtained as described in detail elsewhere.[22] The fibroin solution was loaded into a disposable syringe and injected into 80% methanol aqueous solution in order to obtain the insoluble gel of silk fibroin.[10] After this treatment, the cylindrical gel (30 mm of length, 6.5 mm of diameter) was carefully washed in distilled water and stored in distilled water until MRI measurement.

MR imaging observation was performed as follows. After the gel was set in the vessel, 5 mL of 0.5 M 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPOL) aqueous solution was added. Immediately, the sample was placed in the MRI probe and the data were obtained after one accumulation (5 min). Observation of ^{1}H MR imaging was performed at 25 °C with a JEOL GSX270 (WB) NMR spectrometer equipped with a ^{1}H imaging system (NM-GIM270). The imaging was observed by the spin-echo method[1] (spin-echo time, TE = 29–36 ms; repetition time between two successive individual pulse series applied, T_r = 300–2000 ms). Each MR image was produced by using a two-dimensional Fourier transform (data size = 256 × 256). The image resolution was 0.2 mm, and the slice thickness of observation field was 2 mm.

Spin–lattice and spin–spin relaxation times, T_1 and T_2, of water trapped in the silk fibroin gel were determined according to the usual inversion–recovery method and Carr–Purcell–Meiboom–Gill method, respectively, with a JEOL FX-90Q NMR spectrometer at 90 MHz.

Results and Discussion. Figure 1 shows the time dependence of the MR image of the swollen fibroin gel together with the distribution of the signal intensity of water across the sample (one-dimensional profile) after it was immersed in TEMPOL aqueous solution. Figure 1 (6) is also the image of the gel (diameter = 6.5 mm) in the well-swollen state before immersion in the TEMPOL solution. The distribution of water in the fibroin gel was approximately uniform as shown in the profile of Figure 1 (6) except for the formation of a thin layer of 0.6-mm thickness at the surface. The presence of such a layer is detected from the contrast between the inner and outer parts of the gel, indicating the formation of fine structure with an antiparallel β-sheet form at the surface as a result of the conformational transition from random coil to the β-sheet by contract with methanol.[21]

As shown in both the images and the one-dimensional profiles of the signal intensity of water across the sample (Figure 1 (1–5)), the MR imaging pattern changes drastically with time because of diffusion of TEMPOL molecules, which cause paramagnetic relaxation of the water protons in the gel. Since the gel is in a well-swollen state before immersing it in the TEMPOL solution, this remarkable change clearly indicates the decrease in the ^{1}H NMR relaxation times of water because of the presence of TEMPOL. Figure 2 shows the time dependence of the signal intensity at various positions in the gel as determined from the profile. In the plots, it should be noted that the intensity increases rapidly and then decreases gradually with time. Similar changes were still observed when the experimental conditions were changed, namely, TE = 29–36 ms and T_r = 300–2000 ms. To clarify this nonuniform change of signal intensity, we present the following discussion.

When MR imaging is measured by the spin-echo method, the signal intensity, I, is approximately given by

$$I = k\rho \exp(-\mathrm{TE}/T_2)[1 - \exp(-T_r/T_1)] \quad (1)$$

where k and ρ denote a constant and the proton spin density of water, respectively. The first exponential term is dependent upon both T_2 and TE (2 × pulse interval) and the second term upon both T_1 and T_r. In this experiment, the values of TE and T_r were varied between 29–36 ms and 300–2000 ms, respectively. ρ was constant because of the well-swollen state of water. Therefore, the intensity of the profile strictly includes information on

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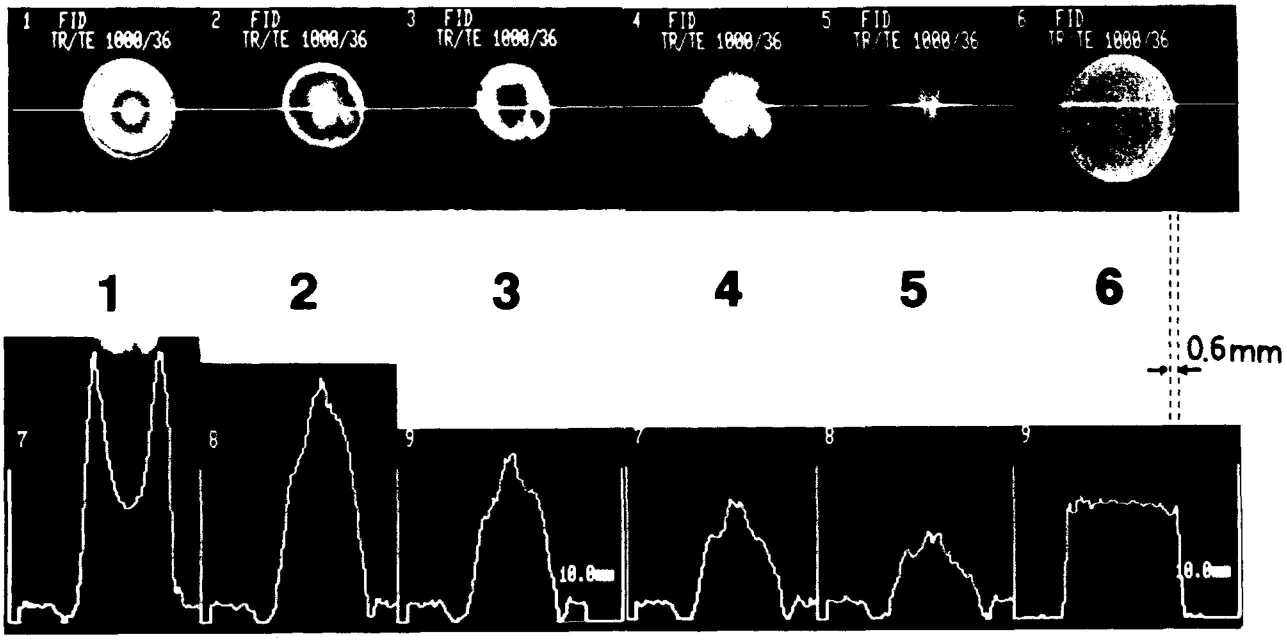

Figure 1. Time coarse of MR imaging of silk fibroin gel after contact with TEMPOL aqueous solution. Spin-echo time = 36 ms. Repetition time = 1000 ms. Observation time (min): (1) 5–10, (2) 15–20, (3) 25–30, (4) 35–40, (5) 45–50, and (6) 0, respectively. Each profile in the picture corresponds to the center line on the image.

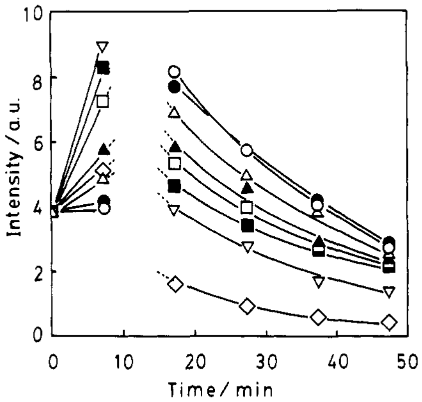

Figure 2. Change of the image intensity as a function of contact time with TEMPOL. These data were calculated from the intensity of the profile at various distances from the surface of the gel. Position from the surface of the gel (centimeters): (○) 0.33, (●) 0.29, (△) 0.26, (▲) 0.23, (□) 0.20, (■) 0.17, (▽) 0.14, (◇) 0.11.

the relaxation times of water molecules rather than the density.

In order to evaluate the change of the image profile in accordance with eq 1, the values of T_1 and T_2 of water were determined as a function of concentration of the TEMPOL from 2 mM to 500 mM (Figure 3). The results indicate that the values of relaxation rates, $1/T_1$ and $1/T_2$, agree within experimental error under these experimental conditions. In addition, a plot of these relaxation rates against TEMPOL concentration yields a straight line. From the plots, we obtained the empirical formula

$$1/T_1 = 1/T_2 = 0.312 + 0.1829[\text{TEMPOL}] \qquad (2)$$

where units of T_1 and [TEMPOL] are seconds and millimolar, respectively. The correlation coefficient was 0.9997. In addition, the equilibrium values of T_1 and T_2 for water in the silk fibroin gel in the presence of TEMPOL approximately agree with those in aqueous solution. Thus, we considered that the change of the signal intensity observed here can be explained as a function of only one relaxation time, T_1 or T_2, with eq 1 by assuming that the frequency dependence of the paramagnetic relaxation is negligible.

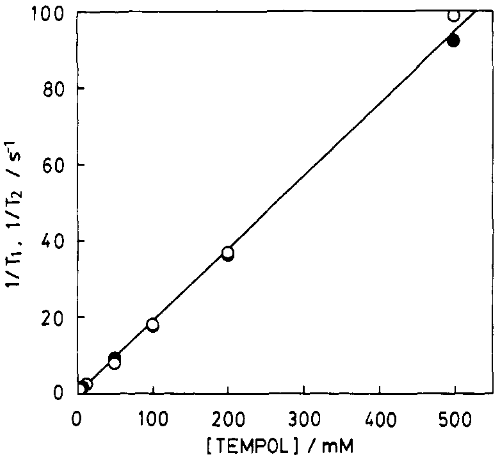

Figure 3. Plot of relaxation rate, $1/T_1$ (●) and $1/T_2$ (○), as a function of TEMPOL concentration.

Figure 4 shows the simulated curves of the signal intensity calculated in accordance with eq 1 under $T_2 = T_1$ at various values of TE and T_r. As shown in Figure 4 (TE = 36 ms, T_r = 1000 ms), the intensities increase rapidly and decrease gradually with increasing $1/T_1$. This tendency agrees with the change of Figure 2 (TE = 36 ms, T_r = 1000 ms). In addition, other observed profiles of the MR image (TE = 29 ms, T_r = 300 or 2000 ms) also changed as shown in Figure 4. Therefore, it is expected that the change of the intensity of the MR image is explained by the decrease of T_1 and T_2 values of water in the silk fibroin gel as a result of the diffusion of TEMPOL.

If one-dimensional diffusion of the TEMPOL is interpreted in terms of Fick's second law,[23] the average diffusion coefficient, D, is calculated with a reduced absorption curve, i.e., a plot of C_t/C_∞ against $(t/L^2)^{1/2}$, where C_t and C_∞ represent concentrations of absorbed TEMPOL in the gel at time t and when equilibrium has been reached in the fibroin gel. In accordance with eqs 1 and 2, the concentration of TEMPOL at various positions L (centimeters) from the surface of the gel was determined. Figure

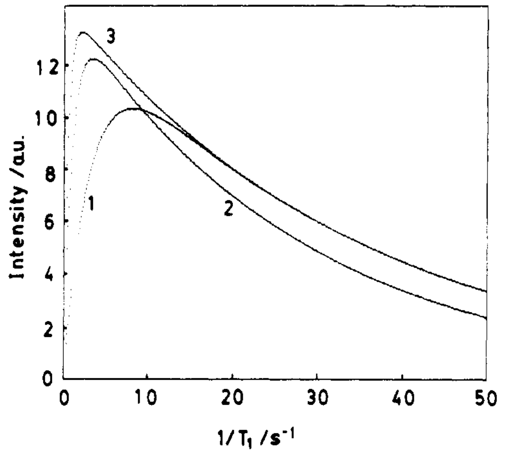

Figure 4. Simulation of the intensity of the MR image as a function of $1/T_1$. Line 1: TE = 29 ms, T_r = 300 ms. Line 2: TE = 36 ms, T_r = 1000 ms. Line 3: TE = 29 ms, T_r = 2000 ms.

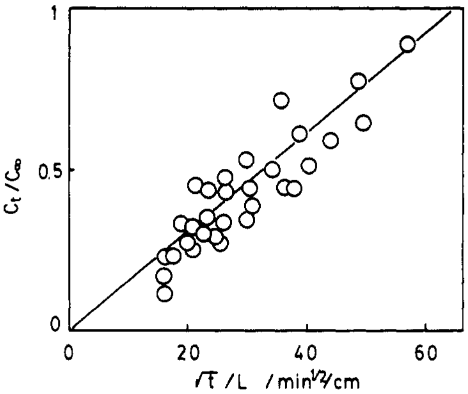

Figure 5. Reduced absorption curve for diffusion of TEMPOL in the silk fibroin gel (correlation coefficient = 0.9577, n = 33).

5 shows the plots of the fraction of the concentration of TEMPOL as a function of $(t/L^2)^{1/2}$. As shown in Figure 5, the plots show a single straight line (correlation coefficient = 0.9577, n = 33). For short times, the average diffusion coefficient, D, is expressed as[23]

$$D = \pi/64/[t/L^2] \tag{3}$$

where $[t/L^2]$ denotes the value of the abscissa when $C_t/C_\infty = {}^1/_2$. From Figure 5, D was calculated as 8.1×10^{-7} cm^2/s. This value agrees within experimental error with the results calculated from imaging data obtained by using the other measurement parameters. In addition, this order of magnitude is consistent with the diffusivity of glucose in the silk fibroin membrane,[11] whose molecular weight is nearly equal to that of TEMPOL.

References and Notes

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Received October 5, 1989
Revised Manuscript Received September 26, 1990

Registry No. TEMPOL, 2226-96-2; water, 7732-18-5.