High Resolution Proton Nuclear Magnetic Resonance Studies of Solute Molecules in the Poly(vinylalcohol) Gel Medium

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It was shown that a pair of spectra is observed for each solute in the medium of poly(vinylalcohol) gels swollen in water. Each of the spectra was assigned as originating from the solute in the PVA gel itself and that in the bulk solution surrounding the gel. It was concluded that the chemical shift difference is primarily explained in terms of the difference in the volume susceptibility of the PVA gel and the bulk solution. It was observed in the case of nicotinamide adenine dinucleotide (NAD) that the chemical shift differences are different for different functional groups. We suggest that this observation reflects a contribution from intermolecular interaction between PVA gel and the NAD molecule.

In a previous work from this laboratory we have shown that in poly(vinylalcohol) (PVA) gels rotational diffusion of nitroxide radicals is strongly dependent upon the pore size of the gels.[1)] In order to characterize in detail the PVA gel as a medium we have measured ^{1}H NMR spectra of a various kinds of solute molecules. The present paper reports an analysis of ^{1}H NMR spectra which are given by solute molecules in the PVA gels. Interactions between solute molecules and the PVA gel will briefly be described using nicotinamide adenine dinucleotide (NAD) which is flexible in shape and is expected to exhibit a pH dependent interaction with the PVA gel.

Experimental

PVA Gels. When an aqueous solution of PVA is irradiated with γ-rays at sufficient dose, the polymer molecules interlink each other, and insoluble gels are formed. It is known that a degradation process rather than cross-linking occurs predominantly when PVA solution is subject to ionizing radiation. When it is irradiated in the presence of water, however, a cross-linking type of reaction occurs, and a gel is formed in the following manner.[2)] Upon γ-ray irradiation, water molecules are turned into free radicals, which react with the protons of PVA. This reaction occurs above the critical concentration; the gel formation does not occur below the critical concentration even upon prolongated irradiation. The critical concentration is approximately 0.36 g/100 ml for PVA with an initial degree of polymerization of 1740.[2)] The gel prepared by a total dose of irradiation below 1×10^6 rads was like a paste. When the total dose was above that level, a rubber-like substance was formed; the gels formed with higher doses of irradiation were of higher density and more elastic. The gels sometimes contain small bubbles, which are mainly H_2 gas developed during the gel formation. The gel was usually colorless, sometimes pale yellow. It is chemically stable. It is stable even when it is heated with boiled water in an autoclave at 150 °C for one hour, and retains its original shape on boiling in 0.1 M HCl solution or in 0.1 M NaOH solution at 100 °C for 3 h.[2)] When it was dried, it shrinked remarkably and turned into a hard colorless or pale yellow substance. When the dried gel is immersed in water, it is swollen back and the original color and shape were restored. However, the color was changed into yellow or light brown when it was swollen in alkaline solution ($\text{pH} > 11$) or in DMSO. In the gel form, the size of the average diameter of the holes in the network can be calculated using

$$\langle l \rangle = 2.5 \times \left(\frac{5.1 \times 10^8 \pm 1.37 \times 10^8 \times C}{R} \right),$$

where R is the amount of the total dose in Rads and C is the concentration of PVA (g/100 ml).[2)] It should be noted that the diameter is represented by a distribution function and that $\langle l \rangle$ does not represent any definite size of the hole. It should rather be, therefore, regarded as only one of the variables which describe the density of the gel. We also defined C_{GEL}, which is the gel concentration

$$C_{\text{GEL}} = \frac{w_{\text{DRY}}}{w_{\text{ORIG}}},$$

where W_{ORIG} is the weight of the original gel and W_{DRY} is that of the dried gel.

Preparation of PVA Gel. PVA, with an average degree of polymerization of 2000, was purchased from Wako Pure Chemicals. Twenty grams of the PVA powder were suspended in 1 L water and stirred while heating until dissolved. Then the solution was stirred another 10 h to be homogenized. Glass tubes filled with the aqueous solution were fixed on a brass stand and were irradiated by γ-ray. The γ-irradiation was performed using three different furnaces A, B, and C at Japan Atomic Energy Institute, Tokai, Japan Atomic institute, Takasaki and institute for Atomic Energy, The University of Tokyo, respectively. Because the gel contracts during formation. as mentioned above, the diameter of glass cylinder must be chosen according to the total dose of irradiation fitting the prepared gel to a 5 mm OD NMR sample tube.

Although all of the glass tubes and the aqueous solution became brown after the irradiation, the gels remained colorless. The gels were washed with distilled water several times, immersed in distilled water and left there overnight to exchange the colored water in the gels for distilled water. Then a 3—4 cm cylinder was cut out from the prepared gel and dried at 50—60 °C *in vacuo* for 20 h. If the temperature is above 70 °C, dried gels become yellow. The dried gel was soaked in sample solutions in 5-mm OD NMR tubes and kept at least for one night at room temperature. Then they swelled back to their original size. The time, which is necessary for PVA gels to be swollen back to the original size, depends strongly on temperature and solvent. In aqueous solution, it took a few hours in hot water, 10 h at 23 °C, 3 d at 10 °C, more than a week in the refrigerator and about a week in DMSO at room temperature. Although the gels which were irradiated using furnaces A and B were homogeneous for NMR measurements, the gels prepared using furnaces C were not suitable for NMR. They often tore to small pieces. Even when they did not, they contained a number of bubbles. In the case of furnace C, homogeneous gels finally could not be

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prepared at any PVA concentration, any degree of polymerization and any dose rate examined. Degassing before irradiation also did not work. A possible reason for the difference between the gels prepared using furnaces A and B and that by furnace C is a difference in the temperature at which irradiation was made. In the case C, the temperature was at 30—50 °C, whereas in the cases of A and B the temperature was maintained at 10—30 °C. Therefore, it may be concluded that the temperature below 30 °C is a necessary condition for obtaining homogeneous gels that are suitable for NMR measurements.

Reagents. NAD was purchased from Kojin (Lot No. 2113) and used without further purification. All other chemicals were of reagent grade.

NMR Measurements. The concentration of NAD was 10—100 mM. The pH was adjusted by DCl or NaOD. All pH values reported are uncorrected meter readings of D_2O solution made with an electrode standardized by using H_2O buffers. PVA gels were prepared by 30 MRads irradiation and $C_{GEL}=23.7\%$. A 3-cm cylinder was cut out from the prepared gels and dried *in vacuo.* The dried gels were immersed in 5-mm OD NMR tubes and kept at least one night at room temperature until they were swollen back to their original size. When the pH was above 8, NAD decomposed within 10 h, and the color of the solution turned into brown. NMR spectra were obtained at 100 MHz with a JEOL PS-100 spectrometer in the correlation mode.[3] Typically 100 transients (409.6 Hz/3.0 s) were accumulated to improve the signal to noise ratio. A Bruker HXS-360 spectrometer was used to apply the magnetic field to the direction which is perpendicular to that in the case of a conventional electromagnet. All chemical shifts are given in ppm relative to internal DSS. The probe temperature was 29 °C.

Results and Discussion

An NAD–PVA gel system gives a spectrum as shown in Fig. 1. Beside a broad signal of the methylene protons of the PVA molecule, all the signals consist of doublets; in each pair, the signal at lower field is broader than that at higher field. For the assignment of each of these signals, a small amount of Fe^{3+} ion was added from the top of the gel in an NMR tube. After addition of Fe^{3+}, each higher field signal instantaneously broadened and became unobservable. In contrast to this, the lower field signals started to show some broadening only after several hours. This result indicates that the signals at higher field arise from the molecules in the solution surrounding the PVA gel (Hereafter the solution will be referred to as the *surrounding solution*) and that the signals at lower field arise from those in the PVA gel.

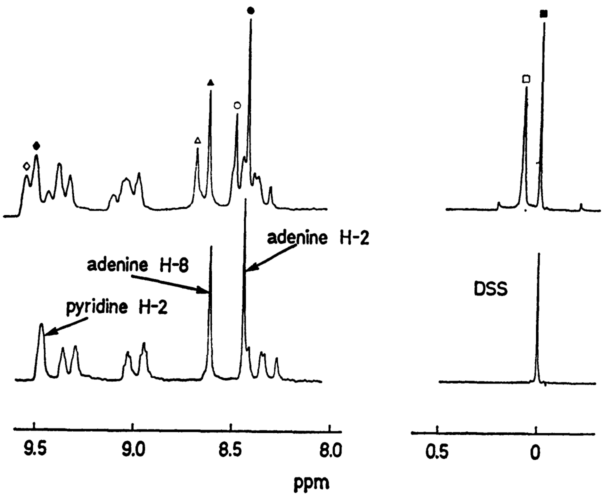

Fig. 1. 100 MHz ^1H NMR spectrum of NAD. The upper and lower spectra correspond to NAD in the PVA gel medium and aqueous solution, respectively. Open and closed symbols indicate signals due to the solutes in the PVA gel and in the surrounding solution, respectively.

Hereafter, the chemical shift for a signal due to the solutes in the PVA gel relative to the corresponding signal of that in the surrounding solution will be simply referred to as a *chemical shift difference.*

It was conformed that with an NMR spectrometer using a superconducting magnet signals due to solutes in PVA gel appear at higher field relative to those of the surrounding solution. The direction of the magnetic field of a superconducting magnet is perpendicular to that of a conventional magnet. It may be concluded, therefore, that the chemical shift difference is primarily due to the difference in the volume susceptibility of the PVA gel and the surrounding solution.[4]

It was observed that the chemical shift differences for each doublet were clearly different. This fact suggests that, in addition to the volume susceptibility effect, a contribution to the observed shift due to an intermolecular interaction between PVA gel and solutes exists at least to some extent. The chemical shift difference ($\Delta\delta$) will be written as

$$\Delta\delta = \Delta\delta_0 + \Delta\delta_1, \tag{1}$$

where $\Delta\delta_0$ is the chemical shift difference which would be given only by the difference in the volume susceptibility of two media, $\Delta\chi_v$, and $\Delta\delta_1$ is the deviation of the observed chemical shift difference from $\Delta\delta_0$.

Two independent methods can be used to estimate $\Delta\delta_0$ and $\Delta\delta_1$. The magnetic field H_n experienced by a nucleus may be given by[5]

$$H_n = H_0 + h_1 + h_2 + h_3 + h_4, \tag{2}$$

where H_0 is the applied external magnetic field; h_1 is dependent upon the shape of the sample tube and the bulk susceptibility of the sample and the sample tube. h_2 is the contribution due to magnetization external to the Lorentz sphere, *i.e.*,

$$h_2 = \frac{4}{3}\pi\chi_v H_0, \tag{3}$$

where χ_v is the volume susceptibility of the molecule. In the case of spherical samples, the contribution $(h_1 + h_2)$ is given for a conventional electromagnet (4) and a superconducting magnet (5), respectively, by[4]

$$h_1 + h_2 = +\frac{2}{3}\pi\chi_v H_0, \tag{4}$$

$$h_1 + h_2 = -\frac{4}{3}\pi\chi_v H_0. \tag{5}$$

h_3 is an intermolecular interaction effect, and h_4 is the intramolecular chemical shift in an isolated molecule. From Eqs. 4 and 5 the observed chemical shift differences $\Delta\delta_{obs}$ for an electromagnet and a superconducting magnet, respectively, are given by

$$\Delta\delta_{obsd} = \frac{2}{3}\pi\Delta\chi + \Delta\delta_1 \tag{6}$$

$$\Delta\delta_{\text{obsd}} = -\frac{4}{3}\pi\Delta\chi + \Delta\delta_1, \qquad (7)$$

where $\Delta\chi = \chi_v^{\text{in}} - \chi_v^{\text{out}}$ is the difference in the bulk susceptibility between in and outside the PVA gel and $\Delta\delta_1$ is the chemical shift induced by the intermolecular interactions. Equations 6 and 7 can be solved simultaneously to give $\Delta\chi$ and $\Delta\delta_1$.

Alternatively, $\Delta\chi$ and $\Delta\delta_1$ can be evaluated by using only one kind of magnet.[5] In this case we assume a mathematically idealized system, *i.e.*, one regards the PVA gel and sample tube as an infinitely long perfect coaxial cylinder. In the absence of sample spinning, the observed NMR spectrum gives two maxima. The separation of the maxima ($\Delta\nu$ (Hz)) is a linear function of the difference in the volume susceptibility of both media which is given by[5]

$$\Delta\chi = 4\pi\nu_0\Delta\chi(a/r)^2, \qquad (8)$$

where r is the radius of inner boundary of the tube, a is that of the PVA gel and ν_0 is a fixed radiofrequency (100 MHz in the present case). With sample spinning, the NMR spectrum of the surrounding solution collapses to a single line. Figure 2 is a spectrum of H_2O in the PVA gel. Spectra A and B are obtained without and with spinning, respectively. The difference in volume susceptibility of a sample can be obtained from this separation. The shape of signals were found to depend strongly on the mapping of heterogeneity in the magnetic field due to imperfections in the PVA gel system. If measurements are carefully performed in the same manner, the values of $\Delta\chi$ obtained in this way become quite reproducible within 0.005 ppm.

In Fig. 3, the chemical shift differences are shown for H_2O, DSS, and glycylglycylglycylglycine(Gly_4) as a function of C_{GEL}. In the case of DSS, $\Delta\delta$ was in excellent agreement with $\Delta\delta_0$ throughout the entire range of C_{GEL} examined. $\Delta\delta$ of DSS depends slightly upon temperature. As the temperature rose, $\Delta\delta_0$ increased; the slope of $\Delta\delta_0/(1/T)$ was quite similar for any sample and for any pH. This fact suggests that $\Delta\delta_0$ depends on temperature but not on pH.

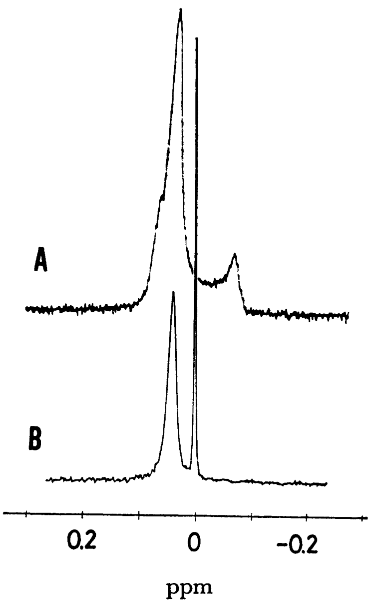

Fig. 2. 100 MHz ^{1}H NMR spectra of water in the PVA gel system. A and B are obtained without and with spinning, respectively.

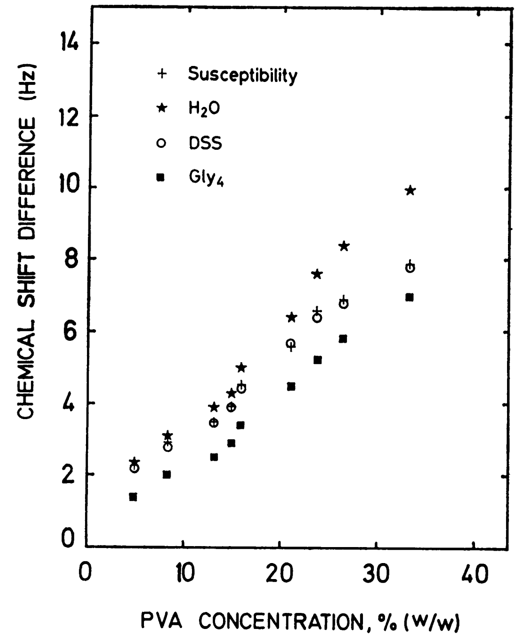

Fig. 3. Chemical shift differences for H_2O, DSS, and Gly_4 *versus* PVA concentration C_{GEL}.

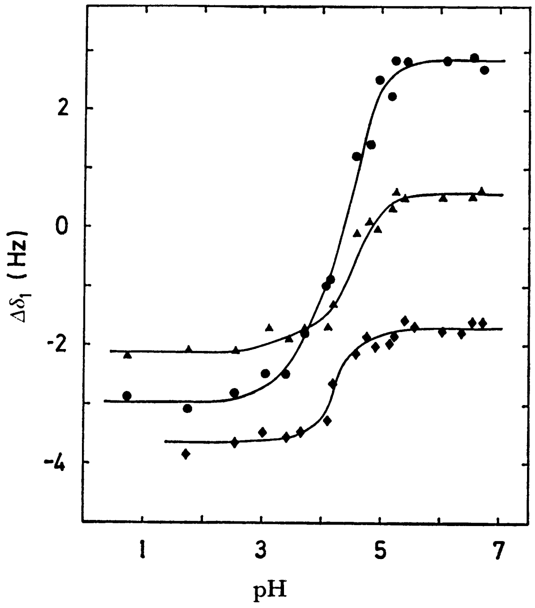

Fig. 4. Titration curves of $\Delta\delta_1$ of NAD.
●: Adenine H-2, ▲: adenine H-8, ◆: pyridine H-2.

As Fig. 4 shows, $\Delta\delta_1$ of the three protons of NAD are dependent upon pH. Each curve gives a quite similar pK_a of approximately 4. The chemical shifts of the adenine signals presumably reflect protonation of *N*-1 at pK_a 3.8.[6] This result may be interpreted in terms of an intermolecular interaction between NAD and PVA gel.

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