NMR Study of the Spatial Effect of Polyacrylamide Gel upon the Water Molecules Confined in It

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Abstract: Analysis of the states of water in polyacrylamide gel and its cross-linked gel was done by the measurements of the spin-lattice relaxation time T_1 and the amount of unfrozen water at −35 °C. It was found that the T_1 of water was reduced and the amount of unfrozen water was increased with an increase in extent of polymerization and cross-linkage of the gels. The results obtained much depend on the nature of the gel, which is characterized by the size of space in the gel.

Introduction

The states of water in polyacrylamide (PAA) gel or other macromolecular gels such as agar, gelatin, and various protein solutions consist of a large amount of "bulk" water and a small amount of "associated" water.[2-5] The former is free state, and the latter is restricted in motion compared to the former. Gels, in general, are constructed of a macromolecular network consisting of a number of small cavities. The water molecules in the cavities are restricted in their motion compared to water molecules in pure water. The restriction for the motional state of water arises from the associated part of water with restricted mobility. In practice, however, the two phases of the water cannot be observed by NMR as the separate signals and the spin-lattice relaxation time T_1 is observed as a single mode. This is for the rapid exchange between the bulk water and the associated water. These systems are well-known to be accounted for by the two-phase model.[6] Therefore, the presence of the associated part of water can be evidenced by the fact that the T_1 of water in the gel becomes shorter than that of the bulk water. On the other hand, the restriction for the motional state of water depends upon the size of the cavity of the gel. The above concept has been extended by several investigators.[7-10] Aizawa and co-workers[7] have referred to the "microspace" effect arising from the cavity of agarose gel. Fujiwara et al.[8,9] have offered the idea of the "molecular space" as the confined space. Recently, we have reported that T_1 of water in the PAA gel becomes shorter than that in acrylamide solutions.[10] The result refers to the fact that T_1 of water is shortened by the restriction of the space which is available for the motion of the water molecule.

We can see the presence of the associated water by NMR of the unfrozen water. The water in the gel is not frozen even if the temperature of the gel sample gets below the freezing point of the bulk water. A broad signal which refers to the unfrozen water appears below ca. −10 °C in the case of the PAA gel.[11] Upon cooling, the associated part of water in the gel is transformed into the unfrozen water. Kuntz and co-workers[12-18] have discussed the amount of associated water in various proteins with the amount of unfrozen water at −35 °C, which gives the most reasonable value for the amount of associated water. As we measure the cooling process of the sample, it is found that the amount of unfrozen water indicates the pronounced temperature dependence. The amount of unfrozen water is not always in accord with that of the associated water. However, it is almost certain that the unfrozen water is at least closely related to the associated water.

In the present paper, the dependences of the gel concentration and condition on the T_1 of water and the amount of unfrozen water in it are examined by use of the PAA gel and the cross-linked PAA gel. On the basis of the results obtained, we discuss the motional state of water in the gels and its restriction effect by the space of the gels, the factor for holding the unfrozen water and its stoichiometric relationships, and the function of the confined space in the gels.

Experimental Section

Recrystallized acrylamide (AA) was purified by sublimation several times under high vacuum and was used for preparation of the linear polymer gel samples. Recrystallized *N,N'*-methylenebis(acrylamide) (MBA) was used for preparation of the cross-linked gel samples. Water used in the present experiment was deionized H_2O and D_2O (E. Merck, 99.75%), which were distilled several times. Polyacrylamide (PAA) gel samples were produced from the aqueous solutions with different concentrations of AA ranging from 0 to 70% by weight. Cross-linked polyacrylamide gel samples were prepared by the mixed solutions of AA and MBA. One series of the samples was the mixed solutions of constant AA (12%) with MBA ranging from 0 to 4%. The other series was the mixed solutions, where total concentrations of the solutions were adjusted to 8% by weight with AA ranging from 8 to 4% and MBA ranging from 0 to 4%. Gelations of these solutions were all done by photopolymerization after addition of a small amount of ammonium persulfate as an accelerator for polymerization.

Our experiments were performed on a JEOL FX-100 NMR spectrometer equipped with the standard variable-temperature unit (JNM-VT-3C), where the temperature could be controlled to an accuracy of within ±2 °C in the present experiment. The spin-lattice relaxation times, T_1's, of water in the gels were obtained by observing the inversion recovery in the repeated 180-τ-90 pulse sequences. The T_1's obtained all were due to the relaxation mechanisms of a single mode.[19,20] These were confirmed by examining the linear relationships between recovering vector of magnetization and time. An amount of unfrozen water was measured in terms of the broad signal of the NMR of the sample, which was first cooled down to liquid nitrogen temperature and then raised to the desired temperature of the experiment (−35 °C). They were estimated from the signal area, compared to that of the standard reference sample which was prepared by adding LiCl and $MnCl_2$ to D_2O (90%).

The line shape and the amount of unfrozen water were affected only slightly by the length of time that the sample was held at any given temperature. The gel sample which got a constant temperature in a period of ca. 10 min was further maintained at this temperature for a period of ca. 1 h. The procedure was necessary in order to obtain good reproducibility with respect to the amount of unfrozen water (the experimental error within ±1%). The frozen gel samples are prone to crack the sample tubes. Then, we routinely used the coaxial tubes made from the standard glass tubes (5 mm o.d.) and the inner tubes made from the stock polypropylene tubes (3.5 mm o.d.) in which the gel samples were placed. The use of the polypropylene tube successfully prevented the cracking of the sample tubes below the freezing point of water.

Results and Discussion

The dependence of T_1 of water in PAA gel on density of the gel, which will be referred to hereafter as the concentration

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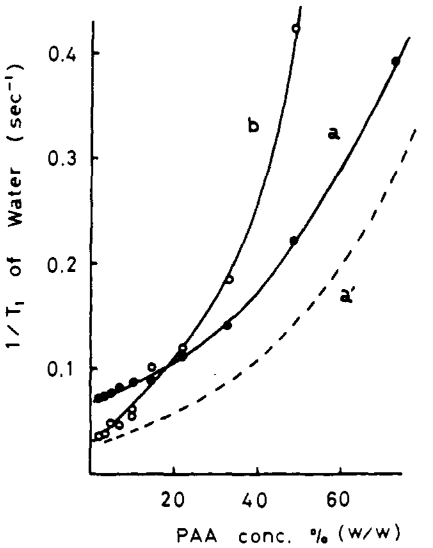

Figure 1. A plot of $1/T_1$ of water in the linear polymer gel (PAA) against concentration of the gel. (a) The curve of $1/T_1$'s of water in the acrylamide (AA) solutions. (b) The curve of $1/T_1$'s of water in the PAA gels. (a') The curve of $1/T_1$'s of water in the AA solutions degassed. The (a') is the estimated curve on the basis of the results in the previous experiment.[10]

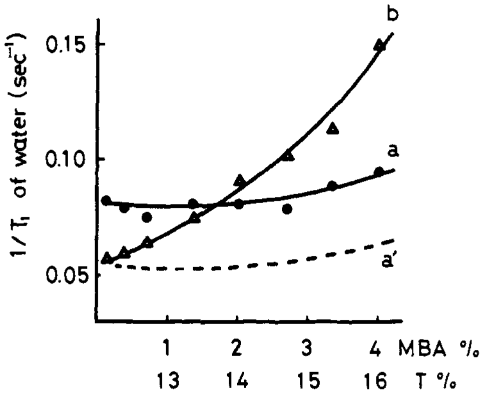

Figure 2. A plot of $1/T_1$ of water in the cross-linked PAA gel against concentration of the gel. (a) The curve of $1/T_1$'s of water in the mixed solutions (AA-MBA). (b) The curve of $1/T_1$'s of water in the cross-linked PAA gels. (a') The curve of $1/T_1$'s of water in the mixed solutions degassed. The (a') is the estimated curve on the basis of the results in the previous paper.[10]

dependence of T_1, has been already studied over the concentration range from 0 to 20% by weight.[10] In the study, it has been clarified that the relaxation of water in the PAA gel is determined by the terms of the nature of the gel and the amount of oxygen molecules being present in the gel. In the present paper, the T_1 of water and the amount of unfrozen water in the PAA gel and the cross-linked PAA gel are measured under the various gel concentrations and conditions.

The plots of $1/T_1$ of water in the PAA gel against density of the PAA gel are shown in Figure 1. The values of $1/T_1$ (curve b) increase with an increase in density of the PAA gel, where the increase is not less than that in the acrylamide solution (curve a) before polymerization. Then, a curve a' is obtained by degassing of the curve a, while the curve b shows the same behavior regardless of degassing or not. Accordingly, the difference between the curve b and a' is assumed essential, because it reflects only the contribution of the nature of the gel, and not the paramagnetic effect of dissolved oxygen. The reason why the degassing of the solutions results in a constant lowering of the T_1 vs. concentration curves whereas that of the gels has no discernible effect on the $1/T_1$ values has been already referred to in the previous paper.[10,21] Namely, the holding of oxygen molecules in the gel is assumed negligible because of the poor solubility and of the scavenging effect in the process of polymerization. The behavior that the trend of the increase of $1/T_1$ for the gel is larger than that for the solution before polymerization can be interpreted in terms of the fact that the water molecules in the gel are restricted in their motion compared to that in the solution before polymerization. The state of water in the gel consists of a large amount of "bulk" water and a small amount of "associated" water, which are accounted for by the two-phase model.[6] The associated water is restricted in motion compared to the bulk water. Therefore, the above behavior of $1/T_1$ of water in the gel depends upon the relative abundance of the two states of water in the gel. The restriction effect for the motional state of water in the gel is attributed to the content of the associated part of water in the gel, that is, it may be concluded that the gel holds a larger amount of the associated part of water than the aqueous solution.

The plots of $1/T_1$ of water in the cross-linked PAA gels against extent of cross-linkage are shown in Figure 2. The values of $1/T_1$ of water in the cross-linked gels (curve b) also increase with an increase in extent of cross-linkage. The degree of the increase is not less than that in the aqueous solutions before polymerization (curve a). Similar to the case of the PAA gels described above, the difference between curves a' and b is significant, and it reflects only the contribution from the extent of cross-linkage, because the contribution from the extent of polymerization of the PAA is nearly constant. In analogy with the PAA gels, the further increase in $1/T_1$ (curve b) for the gels compared to that for the solutions (curve a') before polymerization refers to the fact that water molecules in the gels are restricted in their motion compared to that in the solutions before polymerization. Therefore, the cross-linked gel can hold a larger amount of the associated part of water than the aqueous solution.

For the same concentration change from 12 to 16%, the degree of increase in $1/T_1$ of water in the cross-linked gel ($\Delta(1/T_1)$ = ca. 0.07 s^{-1}) is not less than that in the PAA gel ($\Delta(1/T_1)$ = ca. 0.01 s^{-1}). This also suggests that the cross-linked gel can hold a larger amount of associated water than that in the PAA gel, although both gels hold the same residue concentration.

The spin-lattice relaxation time T_1 reflects the motional state of water. The fact that the value of T_1 is reduced with an increase in extent of polymerization and cross-linkage suggests that the motion of water in the gel is restricted by the gel. The restriction effect is closely related to the gel concentration and condition. The nature and the size of cavity in the gels can be characterized by the gel concentration and condition. Therefore, the cavity of the gel behaves as the space to restrict the motion of water. On the other hand, the gels can hold a larger amount of associated water with an increase in extent of polymerization and cross-linkage. Accordingly, the fact that the gel holds a larger amount of associated water is essentially parallel to the fact that the space of the gel restricts the motion of water in the gel. Thus, the space of the gel should play an important role in not only the restriction effect for the motional state of water in the gel but also in holding the associated water in it.

Two different approaches have been used to describe the associated water, one the relaxation time T_1 described above and the other unfrozen water described below. The former does not always give direct evidence for the presence of the associated water, for T_1 arising from only the associated part of water cannot be observed. The latter is a useful method to investigate the associated water, for the associated part of water is directly transformed into the unfrozen water, although the amount of unfrozen water depends upon temperature.[11] In the

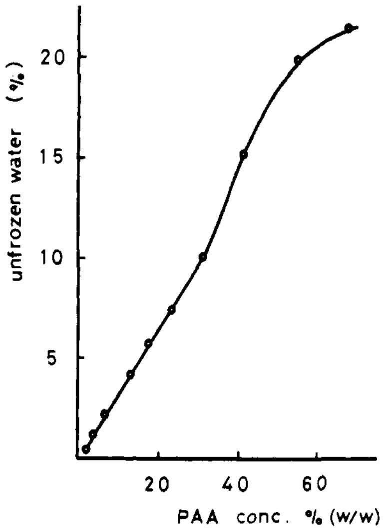

Figure 3. A plot of the amount of unfrozen water in the polyacrylamide gels against concentration.

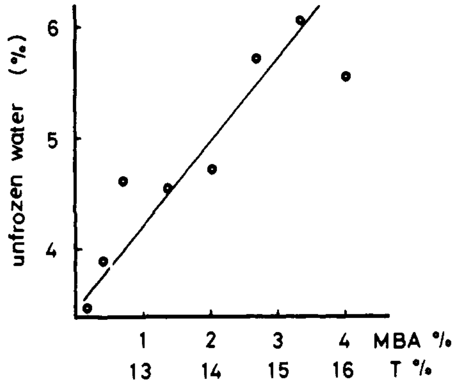

Figure 4. A plot of the amount of unfrozen water in the cross-linked PAA gels against concentration.

following, some discussions will be given on the nature and the stoichiometry of the unfrozen water.

Figure 3 shows the plots of amount of unfrozen water in the PAA gel against concentration of the PAA gel. The amount of unfrozen water in the PAA gel increases linearly with concentration of the PAA up to ca. 30%. In this range, it is found that the gel sample holds 0.3 g of unfrozen water per g of solute, namely, it traps 1.2 water molecules per monomer unit in the PAA gel.

Figure 4 shows the plots of amount of unfrozen water in the cross-linked PAA gel against concentration of the cross-linked PAA gel. The amount of unfrozen water in the cross-linked PAA gel increases linearly with an increase in content of MBA. Although Figure 4 shows some scatter, the trend of the linear relation is probably real. In this case, it is found that the gel sample holds ca. 0.43 g of unfrozen water per g of solute, namely, it traps ca. 1.7 water molecules per monomer unit in the cross-linked PAA gel. On the other hand, it is found that the increase of the unfrozen water in the cross-linked PAA gel is 2.5 ± 0.5% for the concentration change from 12 to 16%, while in the PAA gel it is 1.4 ± 0.1% for the corresponding concentration change. Therefore, the cross-linked PAA gel also holds a larger amount of unfrozen water than that of the PAA gel, although both gels have the same residue concentration.

It should be emphasized that no unfrozen water can be found in the aqueous solutions before polymerization in both the PAA gels and the cross-linked PAA gels. The AA molecules in the aqueous solutions before polymerization cannot hold any associated water as a result of weak hydration; therefore, the unfrozen water cannot be observed. Once the framework of the polymer gel is built up by polymerization, the weak hydration to each residue on the gel polymer is additively concentrated, and hence the strongly associated water cooperatively appears in the gel. Therefore, the unfrozen water can be evidenced. In other words, the effect of the space of the gel occurs, where the water molecules confined in the space of the gel show the behavior of the unfrozen water.

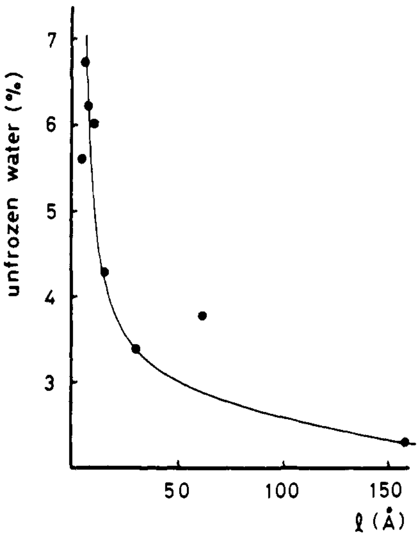

Figure 5. The relationship between the amount of unfrozen water and the length (l) between the cross-linkages in the cross-linked PAA gels. The cross-linked gels are produced from the mixed solutions of constant AA (12%) with MBA (0–4%).

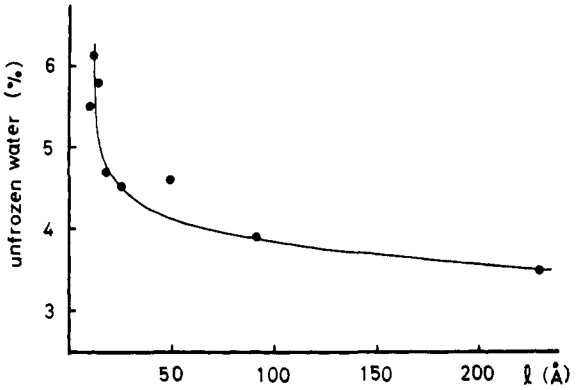

Figure 6. The relationship between the amount of unfrozen water and the length (l) between the cross-linkages in the cross-linked PAA gels. The cross-linked gels are produced from the mixed solutions of AA (8–4%) with MBA (0–4%).

More detailed discussion on the spatial effect will be done by examination of the relationship between the amount of unfrozen water and the size of space of the cross-linked PAA gel. An average length (l) between the cross-linkages is estimated by assuming the length ($l_0 = 2.543$ Å) for every other carbon of polyethylene, whose structure is planar zigzag. The plot of the amount of unfrozen water against l is shown in Figure 5. When the length l is less than 20–30 Å, the amount of unfrozen water indicates an abrupt increase. Similar behavior is found in the experiment with another series of the cross-linked PAA gels whose total concentrations are all kept at 8% as is shown in Figure 6. Although Figures 5 and 6 show some scatter, the above trend is probably real. The fact that the ability of the space of the gel to hold the unfrozen water increases abruptly below 20–30 Å suggests that there is a critical size in the space. The critical length of l = 20–30 Å is in fair accord with the result of the behavior of ESR spectra of Cu^{2+} in poly(vinyl alcohol) by Fujiwara.[8]

In general, the following factors are expected to play an important role in holding the unfrozen water. The first factor is that molecules must be necessarily polymer. Exceptions are glycerine and poly(ethylene glycol), which can hold the unfrozen water. The cases of the exceptions could be attributed to the fact that the two molecules possess a unique ability to form strong hydration. The second factor is that sample molecules should hold hydrophilic substituents such as -OH, -COOH, >CO, -CHO, $-CONH_2$, >NH, etc. The amount of unfrozen water remarkably depends upon the amount and the nature of the substituents. The third factor is that sample molecules hold structural specificity. This is not studied yet in the present investigation. This will be seen in the cases of the three-dimensional structures in biological systems such as proteins, for example, "helix-coil", "nature-denatured", and "sol-gel" transformations. The final factor is that sample molecules hold the spatial effect as mentioned in the present paper. This factor is assumed to play often a most significant role in holding the unfrozen water.

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Generation of Spirodihydroaromatic Anions. Chemistry and ^{13}C NMR

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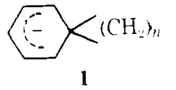
Abstract: A series of salts of 4,4-spiro-1,4-dihydropyridines, **12b,d–h,** has been prepared by cleavage of the corresponding 4,4-spiro-*N*-carboethoxydihydropyridines. NMR investigation shows the salts, except for **12h,** to contain conjugated anions largely unperturbed by counterion and by the nature of the spiro structure about C_4. It is concluded that there is no evidence for interaction between the rings in anions **12b,d–g.** In the case of **12h** the ^{13}C NMR data imply quite a different charge distribution compared to the precursor urethane which was already shown to have any minor interactions between the rings. Urethanes which failed to give stable spiro anions underwent opening (spirocyclopropyl) or cleavage reactions (spirocyclobutyl) as a result of strain in the 4,4 ring. It is concluded that the series of anions studied fails to show evidence for nonclassical structures, and the stability of the anions can be ascribed to the electron-deficient character of pyridine.

While spirodihydroaromatic cations, such as phenonium ions, long postulated as intermediates or transition states in carbonium ion rearrangements,[1] have now been solidly authenticated with spectral techniques,[2,3] the corresponding anions, **1**, have remained comparatively neglected. Such species, first proposed to account for the course of carbanionic rearrangement reactions,[4,5] were later circumstantially implicated from labeling studies[6] and electrophile capture reactions, using CO_2.[7] Thus, for example, the isolation of spiro acid **2** was concluded to imply the short-lived intermediacy of **3.** To unequivocally establish the existence of spirodihydroaromatic anions requires their direct spectroscopic observation. We chose to exploit the electron deficiency of pyridine[8] to stabilize a spiro anion; thus, ^1H NMR was utilized to identify **4** in tetrahydrofuran (THF) solution. Simultaneously, **5** was authenticated in similar fashion.[10]



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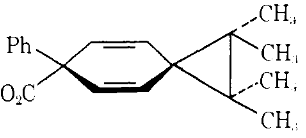

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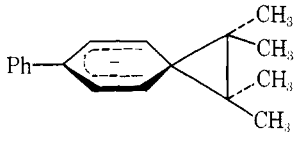

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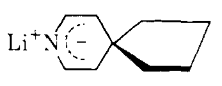

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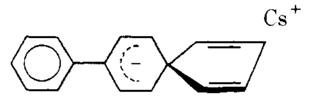

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Using the convenient methods developed to produce **4,**[9,11] we now report on the preparation and properties of a whole

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