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Properties of Water in Macromolecular Gels. IV. Proton Magnetic Resonance Studies of Water iu Macromolecular Gels

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Electrochemical and dilatometric studies have indicated that at least three states of water exist in macromolecular gels. The properties of each state of water in gels such as agarose gels were investigated by high-resolution and broadline PMR over the temperature range from +50 to −60°C. The linewidth of water protons in gels was found to depend markedly on the water content of gels, and the linewidth *vs.* water content curve gave two inflection points. One of the inflection points can be attributed to the so-called bound water, but the other cannot be explained unless three states of water are assumed in gels. Three kinds of transition temperatures were confirmed by anomalous changes in the PMR linewidth below 0°C. The so-called bound water exhibited no transition temperature between 0 and −20°C, and free water gave the transition temperature at 0°C. Moreover, the transition temperature of a quantity of water was distributed in the range from 0 to −20°C. On the basis of the transition temperature, the properties of water in macromolecular gels are discussed.

A solid-like state of a biopolymer adsorbing a small amount of water and an aqueous solution of biopolymer have been taken as the samples in most of the investigations of the properties of water in biopolymers, such as proteins, starch, and cellulose.[1] These two states of biopolymers investigated, however, seem to be somewhat different from that of native biotissues, which include a large quantity of water in their network. Macromolecular gels, such as polysaccharide and protein, have been used as the samples during the present investigation. Especially the results on agarose gels will be described in this report.

Two states of water, a so-called bound water and free water, are accepted to be present in macromolecular gels. Electrochemical[2,3] and dilatometric studies of the properties of water in macromolecular gels[4] have made it clear that free water in macromolecular gels should be classified into at least two groups. Furthermore, the properties and states of water in agarose gels were investigated by PMR in this investigation.

The nuclear magnetic resonance linewidth is known to be related with the spin-spin relaxation time, T_2, indicating the motional state of a nuclear spin.[5] Therefore, the motional state of water can be estimated by means of the PMR linewidth of water protons. In recent years, the technique of nuclear magnetic resonance has been used with some success to characterize distinct types of the molecular motion of water adsorbed on biopolymers.[6-10] Several PMR studies of water in agar have been carried out. Woessner[11] used pulse PMR to study the temperature hysteresis in agar sol-gel transitions of both the agar macromolecules and water in the agar–water system; his results indicate that the major portion of the water molecules (in the agar–water system) is not in an "ice-like" or modified state. These applications of PMR, however, have been limited to investigations on the state of water adsorbed by macromolecules containing either small amounts of water or a large quantity of water. In order to obtain additional information concerning molecular motions of water in macromolecular gels, the high-resolution and broad-line PMR spectra of water in agarose gels were measured over the full range of water content from 0 to 100% by weight. Additionally, the solid-liquid transition of water in gels was investigated by studying the temperature dependence on the PMR linewidth of the water proton in the agarose–water system to clarify the properties and the states of water in gels.

Experimental

Materials. The agarose for immuno-electrophoresis use was purchased from the Nakarai Chemicals Co., Ltd., and was assayed for its ash content by the atomic-absorption analysis method. The agarose contained Mg 0.04, Ca 1.01, K 0.09, and Na 1.75 mg/g dry agarose.

A series of samples was obtained by varying the mixing ratio of water and agarose; they were sealed in a standard PMR sample tube (5 mm O.D.). The water content of the sample was determined by drying it at 105°C for 24 hr and by then weighing the dried matter.,

PMR-spectra Analysis. The proton NMR spectra were obtained using a JNM–C–60HL high-resolution NMR spectrometer and a MW–40 broad-line NMR spectrometer operating at 60 MHz and 40 MHz respectively, with an external NMR lock system. Since the signals were wider than those normally seen in high-resolution work, it was necessary to operate at optimum rf power levels, just below signal saturation. The spectrometer was equipped with a variable-temperature controller which maintained the sample within ±0.5°C of the desired temperature. The temperature

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[2] J. Mizuguchi, M. Takahashi, and M. Aizawa, *Nippon Kagaku Zasshi* **91**, 723 (1970).

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of a sample was calibrated by means of a copper-constantan thermocouple placed inside the sample tube. The linewidth of the spectra was expressed in terms of half linewidth, *i.e.*, the linewidth at half of the maximum peak height.

Results

Broad-line PMR Spectra of Agarose Gels. The broad-line PMR spectra of agarose gels were measured in order to distinguish the signal of the water proton from that of the agarose protons. For the agarose–water system containing 10% of water by weight, the spectra are shown in Fig. 1, with the abscissa expressed in Gauss units. Two components, namely, a broad one which can be attributed to the agarose proton and a narrow one attributable to the water proton, were detected, though the broad component disappeared in the (b) spectrum. The linewidth for the agarose proton in the (a) spectrum is evaluated as approximately 9 gauss. Therefore, it is reasonable to assign the signal in high-resolution PMR spectra to the water proton.

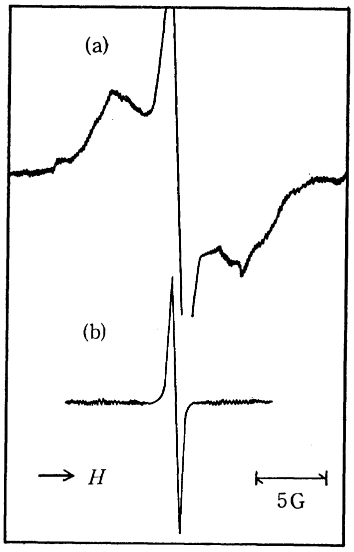

Fig. 1. Broad line PMR spectra of agarose containing 10% of water.
(a): Gain 1.0×100, Response 2, Mod. width 1G; (b): Gain 1.0×10, Response 1, Mod. width 1G, (25±0.5°C).

PMR Linewidth of water in Various Water Contents of Agarose Gel. PMR measurements showed that the line shape and width of the water proton are independent of the orientation of the gels in the magnetic field; this means that there is no preferential orientation of water molecules with respect to the gel structure when this technique is used.

The PMR spectra for agarose gels containing 98 and 80% water at room temperature are illustrated in Fig. 2; the spectra are attributed to protons of water in agarose gels.

The PMR linewidth of water in agarose gels was measured for the full range of water content from 0 to 100%. The results are presented in Fig. 3, where the linewidth is given as the linewidth at half of the maximum peak height. In the water-content range from 100 to 60% and from 30 to 0%, the linewidth was found to increase with a decrease in the water content. On the other hand, the linewidth of water protons is seen to be independent of the water content in the water-content range approximately from 60 to 30%.

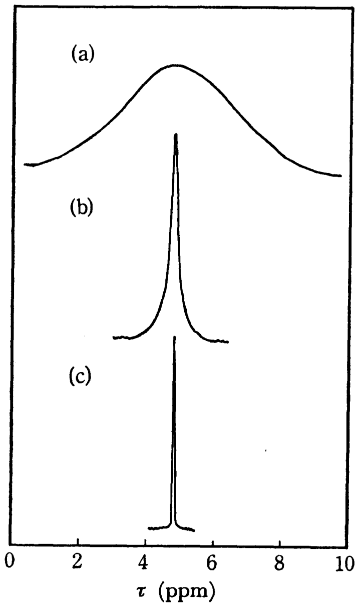

Fig. 2. PMR spectra [of water in agarose gels; water content.
(a): 80%, (b): 98%, (c): pure water (H_2O), (25±0.5°C).

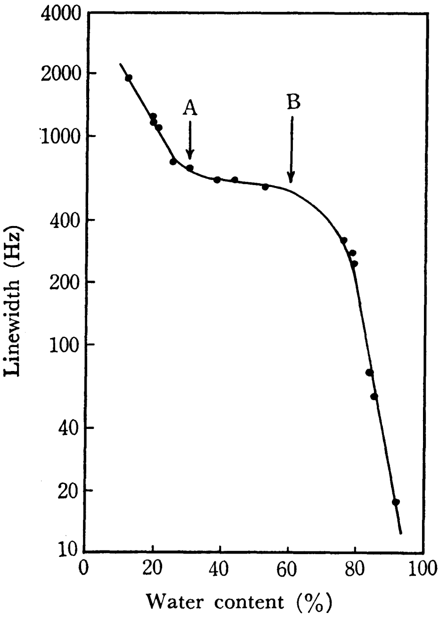

Fig. 3. Water content dependence of the linewidth of water proton in an agarose gel (25±0.5°C).

Temperature Dependence of the PMR Linewidth of Water at Lower Temperatures. It has been known that ordinary water exhibits a transition at 0°C, which is accompanied with a marked change in the NMR linewidth of its proton. On the other hand, the water in gels is considered to give a transition temperature different from that of the ordinary water.

The transition temperature at lower temperatures was determined in accordance with the relationship between the temperature and the NMR linewidth of the water proton.

Agarose gels with various amounts of water were classified into three groups, A, B, and C. Group A contains less than approximately 30% of water, most of which may be dominantly contributed to a motional state of the so-called bound water. The water content of Group B ranges from approximately 30 to 60%, while that of Group C is above about 60%.

The PMR spectrum for each sample was at first measured with an elevation of temperature in the range from 0 to 50°C, and then in the range from −60 to 0°C after a sufficient precooling of the sample in liquid nitrogen.

The PMR spectra of a sample containing 78.5% of water (by weight) at various temperatures are shown in Fig. 4. Each linewidth increases markedly with a decrease in the temperature.

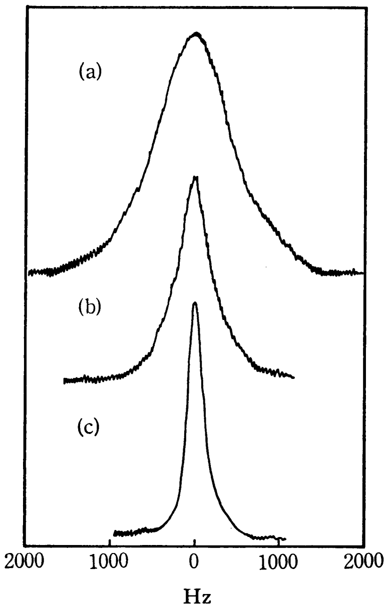

Fig. 4. PMR spectra of agarose gel (water content 78.5%); (a): −50°C, (b): −14°C, (c): 25°C.

The temperature dependence of the linewidth for Group A is shown in Fig. 5, where the abscissa represents the reciprocal of the absolute temperature (1/°K) and where the coordinate represents the half-linewidth (Hz). The PMR linewidth for Group A increases linearly with a decrease in the temperature up to approximately −20°C ($1/T=3.95\times10^{-3}$); the increase in the rate is found to change slightly at this temperature. In other words, an inflection point (T_γ) is found in Fig. 5.

The temperature dependence of the linewidth for Group B is presented in Fig. 6, and that for Group C, in Fig. 7. In contrast with Group A, the linewidth for Group B decreases with a decrease in the temperature up to approximately −20°C ($1/T=3.95\times10^{-3}$), though the inclination is changed in the range approximately from 0°C to −20°C ($1/T=3.66$ to 3.95×10^{-3}). On the other hand, the linewidth for Group C was found to change markedly at 0°C ($1/T=3.66\times10^{-3}$) in addition to the marked change at −20°C ($1/T=3.95\times10^{-3}$).

Discussion

We expected to detect one inflection point in the linewidth-water content curve, because two states of water are generally considered to exist in gels. We found, however, two inflection points, A and B, on the curve, as is shown in Fig. 3. The water content at the A inflection point can be attributed to the amount of so-called bound water in an agarose gel. Whereas the B inflection point is a new finding. From the independence of the linewidth of the water content, it seems reasonable to suppose that the water molecules in agarose gels containing 30 to 60% of water are in comparable motional states, for the curve is flat in the water-content range from 30 to 60% shown in Fig. 3.

On the other hand, the results obtained in previous electrochemical and dilatometric studies[2–4] can not be explained without supposing three states of water

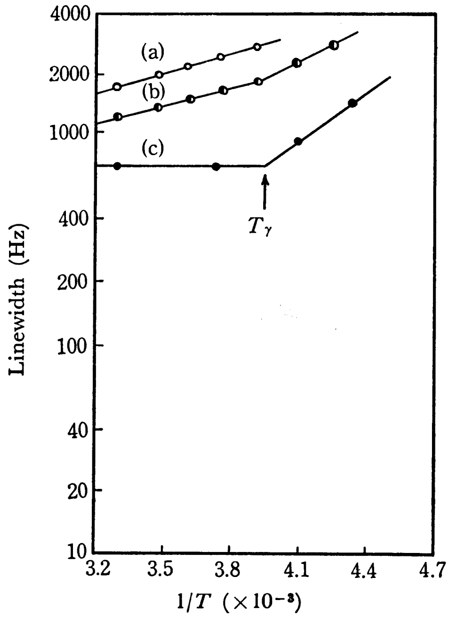

Fig. 5. PMR linewidth of water in agarose gels (Sample belonging to Group A) as a function of temperature. Water content; (a): 12%, (b): 19%, (c): 26%.

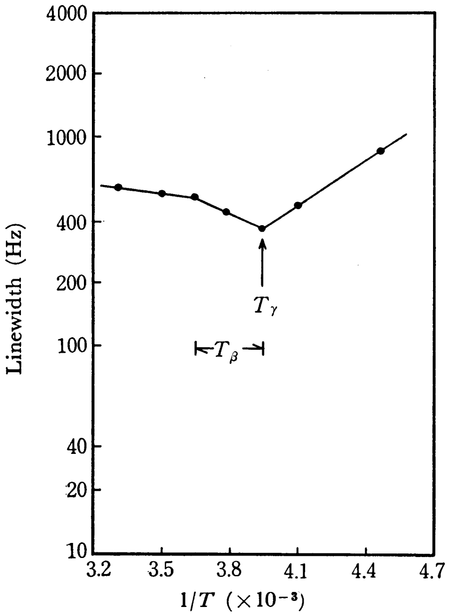

Fig. 6. PMR linewidth of water in agarose gel containing 48% of water (Sample belonging to Group B) as a function of temperature.

in gels.

Therefore, it can be concluded that there are at least three states of water molecules in macromolecular gels. These states of water may be named W_1, W_2, and W_3, of which W_1 and W_2 are comparable to conventional free water, and W_3 to the so-called bound water.

The transition of water is expected to be accompanied by a marked change in the PMR linewidth. Each agarose gel was found to exhibit marked changes in PMR linewidth at lower temperatures, as is shown in Figs. 5, 6 and 7. In addition, these changes depend on the water content of agarose gel, as is presented in Fig. 8. Therefore, the marked changes in Figs. 5, 6 and 7 may be presumed to be caused by the transition of water in gels.

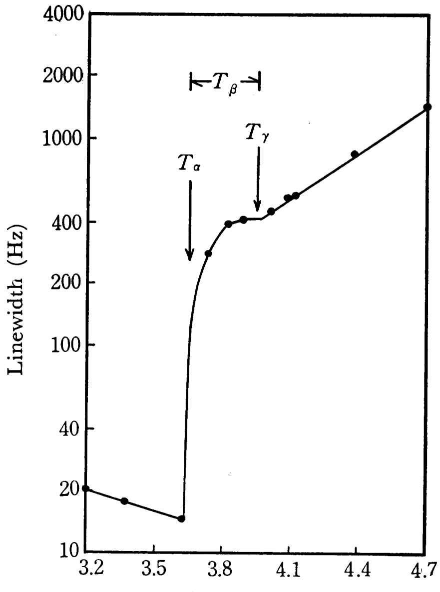

Fig. 7. PMR line width of water in agarose gel containing 92% of water (Sample belonging to Group C) as a function of temperature.

When these inflection points for Group C are named T_α, T_β, and T_γ respectively, as is presented in Fig. 7, the two in Fig. 6 may be T_β and T_γ, and the one in Fig. 5 may be T_γ, because the corresponding temperatures at the inflection points agree with each other.

The electrochemical studies have shown that the transition temperature of water in a gel was distributed from 0°C to approximately -20°C, but no transition temperature for a gel containing a small amount of water was confirmed at lower temperatures. Those results agree with those of nuclear magnetic resonance studies. As is shown in Fig. 7, the transition temperature is distributed from T_α to T_γ, *i.e.*, in the range of T_β. On the other hand, an agarose gel containing a very small amount of water exhibits no transition temperature.

On the basis of these results, the properties of W_1, W_2, and W_3 in gels can be presumed to be as follows.

As is shown in Fig. 7, the linewidth changes abruptly at T_α; W_1 may exhibit a transition between the liquid and the solid states corresponding to ordinary water at 0°C ($1/T=3.66\times10^{-3}$), *i.e.*, free water, though W_1 in gels is hindered from flowing out.

W_3, corresponding to the so-called bound water, may be oriented on agarose by the interaction between the H_2O molecule and the OH group of agarose, since the W_3 content almost agree with the OH-group content of agarose, which is calculated according to the proposed structure[12] of agarose shown in Fig. 9. Therefore, W_3 should exhibit solid-like behavior if such interaction is strong. Since the linewidth of W_3 is of the order of 1000 Hz, W_3 is supposed to be in a solid-like state, even at room temperature, due to the strong interaction between the H_2O molecule and the OH group of agarose. On this supposition, it is reasonable that the transition temperature for W_3 cannot be confirmed in the range of temperature investigated here.

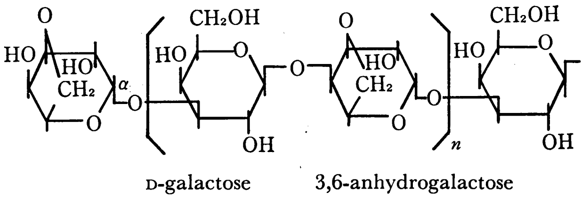

Fig. 8. Structural formula of agarose[12]

The properties of W_2 have not yet been clarified except that the linewidth of W_2 is several hundred Hz, and except that the transition temperature of W_2 may be distributed from 0°C to approximately -20°C. As one would expect, the molecules of water in gels are hydrogen-bonded; W_1, W_2 and W_3 cannot be sufficiently discriminated, but each of them shows dominant properies related to the restriction of water in the potential fields which exist in cavities of the gel. The properties of W_2 described above, however, indicate that W_2 is more fixed than W_1, but more mobile than W_3. It should be emphasized that W_2 is presumed to play an important role in the structure and properties of macromolecular gels because its properties are intermediate compared to those of W_3 and W_1. The detailed properties and the states of W_2 are under the investigation.

Conclusion

The properties of three states of water in agarose gels were investigated by high-resolution PMR over the temperature range from +50 to -60°C.

The transition temperature for water in agarose gels was confirmed to be distributed from 0°C to approximately -20°C by the anomalous temperature-dependence of the PMR linewidth. The transition temperature at 0°C is attributed to that of W_1, and the transition temperature of W_2 may be distributed from 0°C to approximately -20°C. On the other hand, W_3 may give no transition tempersture in this temperature range. Therefore, W_1 may be ordinary water, though it is prevented from flowing out. W_2 may be more fixed than W_1, but more mobile than W_3, and W_3 may be in a solid-like state even at room temperature due to a strong interaction with the OH group of agarose.

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