Properties of Water in Macromolecular Gels. V. Anomalous Temperature Dependence of the Nuclear Magnetic Resonance Line-width of Water in Macromolecular Gels

Masuo AIZAWA, Shuichi SUZUKI, Teruo SUZUKI,* and Hiroshi TOYAMA*
Research Laboratory of Resources Utilization, Tokyo Institute of Technology, Meguro-ku, Tokyo
**Japan Electron Optics Laboratory Co., Ltd., Akishima, Tokyo*

(Received March 22, 1972)

The line-width of the nuclear magnetic resonance (NMR) signal gets narrow, in general, with an increase in the temperature. In contrast, the NMR line-width of the water proton in agarose gels was found to increase with an increase in the temperature up to about 30°C. As for the other polysaccharide gels, such as curdlan-type polysaccharide, and κ- and λ-carrageenan gels, the NMR line-width of water increases with an increase in the temperature. The anomalous temperature dependence of the line-width was most marked in agarose gels than in other polysaccharide gels. The close relationship between the gel network, which consists of small cavities (microspace) of the micelle, and the magnitude of the anomality was revealed, this anomalous effect was named the "Micro-Space Effect."

The line-width of the nuclear magnetic resonance (NMR) signal is known to become narrow as a result of the activation of molecular movement with an increase in the temperature, namely, motional narrowing.[1] In contrast, the present authors have found that the NMR line-width of the water proton in agarose gels containing more than 30% of water became wide with an increase in the temperature. Thus, the molecular motion seems to get bounded with an elevation in the temperature.

It was shown in previous papers[2–3] that water in polysaccharide gels is markedly affected by the three-dimensional framework of the polymer. The thermal expansion of water, for example, was found to be extremely obstructed in gels. The double-helix model, in which the two chains of each pair are parallel and are twisted around each other, has been proposed for the possible structure of such polysaccharides as agarose and carrageenan. The proposed model[4–5] suggests a mechanism for the formation of gels when dilute aqueous solutions of these polysaccharides are cooled: chain segments associate in double helices to crosslink the chains in a three-dimensional framework which is then responsible for the shape and strength of the gel. Subsequently or simultaneously, the double helices could combine into larger aggregates. There is much evidence in support of such mechanism. Furthermore, the effect of structural variations on gel properties may be explained in terms of the model for junction zones. Therefore, the temperature effect on the properties of water may depend on the properties of the junction zones and the framework. The small cavities in the three-dimensional framework of polysaccharide gels can be called the micro-space. Water is included in this micro-space. Therefore, the three-dimensional framework in gels was presumed to be responsible for the anomalous temperature dependence mentioned above. In other words, the anomalous temperature effect was supposed to be related with the micro-space in gels.

Agarose gels are known to be quite different from other polysaccharide gels, such as κ-carrageenan, λ-carrageenan, and curdlan-type polysaccharide gels, in their gel properties. A different anomalous temperature dependence of the NMR linewidth can be expected for these polysaccharide gels. The NMR linewidth was measured for these polysaccharide gels compared with agarose gels in the temperature range from 0 to 60°C.

Experimental

Materials. The agarose, κ-carrageenan, λ-carrageenan and curdlan-type polysaccharide gels used in this study were prepared with distilled water treated with ion-exchanger resins, and were sealed in a standard NMR sample tube (5 mm O.D.). Curdlan-type polysaccharide,[6] a bacterial gel-forming β-1, 3-glucan, which was supplied by Takeda Chemical Industrials, Ltd., was obtained from a culture filtrate of a mutant strain (NTK-U, IFO 13140) of *Alcaligenes faecalis var. myxogenes strain* 10C 3K. These samples were assayed for their ash content by the atomic absorption method. The analytical results on the ash content are shown in Table 1. The water content of the sample was measured by drying it at 105°C for 24 hr and by that then weighing the dried matter after the NMR measurement.

NMR Spectra. The proton NMR spectra were obtained using a JNM-C-60HL high-resolution NMR spectrometer with an external-lock system. Since the signals were wider than those normally seen in high-resolution measurement, it

TABLE 1. ASH CONTENTS OF THE SAMPLES (mg/g DRY MATTER)

	Mg	Ca	Na	K	Fe,Cu
Agarose	0.04	1.01	1.75	0.09	—
κ-Carrageenan	0.23	8.11	6.20	41.3	—
λ-Carrageenan	0.77	15.98	18.79	18.80	—
Curdlan type β-1,3-glucan	0.03	0.06	0.26	2.03	—

---

[1] N. Bloembergen, E. M. Purcell, and P. V. Pound, *Phys. Rev.*, **73**, 706 (1948).

[2] S. Suzuki, M. Aizawa, and M. Takahashi, *Kobunshi*, **20**, 674 (1971).

[3] M. Aizawa and S. Suzuki, This Bulletin, **44**, 2967 (1971).

[4] D. A. Rees, I. W. Steele, and F. B. Willismson, *J. Poly. Sci., Part C*, **28**, 261 (1969).

[5] D. A. Rees, *Advan. Carbohydrate Chem.*, **24**, 267 (1969).

[6] T. Harada, A. Misaki, and H. Saito, *Arch. Biochem. Biophys.*, **124**, 292 (1968).

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

Results

(1) Agarose Gels. The signal of the agarose proton is much wider than that of the water proton, as has been shown previously by broad-line NMR. Therefore, the proton signal in the high-resolution NMR spectra of agarose molecule can be distinguished from that of water.

The temperature dependence of the linewidth of the water proton in agarose gels was investigated in the range from 0 to 60°C at intervals of 5°C by high-resolution NMR. The spectra are illustrated for an agarose gel containing 92% of water at 10, 29, and 53°C in Fig. 1. The line shape remains evidently unchanged in the above temperature range, though the linewidth varies.

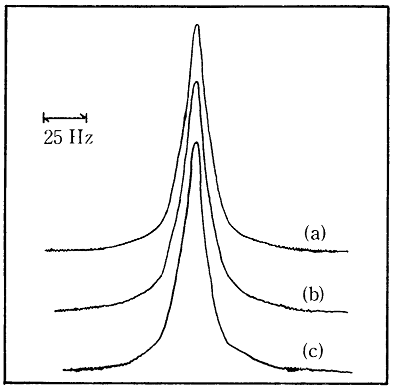

Fig. 1. NMR spectra of agarose gel containing 92% of water at (a) 53°C, (b) 29°C, and (c) 10°C.

The temperature dependence of the linewidth of the water proton in an agarose gel containing 92% of water is shown in Fig. 2. The linewidth was found to increase with an elevation of the temperature in the range from 0 to 35°C. In contrast, at temperatures higher than in the above range, the linewidth decreases as the temperature increases.

The linewidth temperature curves for agarose gels containing 79 and 72% of water are shown in Figs. 3 and 4. An anomalous temperature dependence was observed similar to that of the agarose gel containing 92% water.

(2) κ-Carrageenan and Curdlan-type Polysaccharide Gels. The gel properties of κ-carrageenan and curdlan-type polysaccharide gels are quite different from those of agarose gels. κ-Carrageenan and curdlan-type polysaccharide gels are very flexible, though the gel is weak in its gel strength. If the above anomalous temperature dependence of the line-width in agarose gels is related with the gel framework, different temperature dependences can be expected.

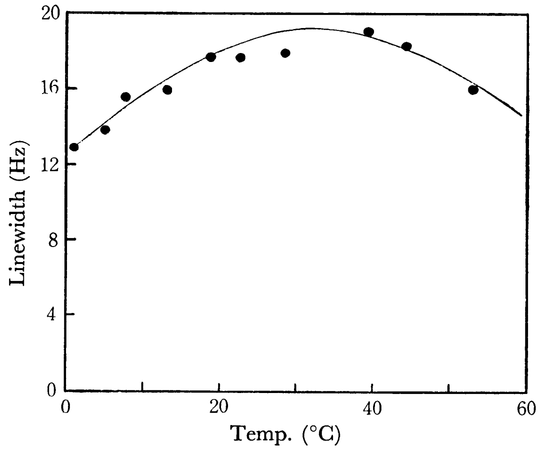

Fig. 2. Temperature dependence of the linewidth for agarose gel containing 92% of water.

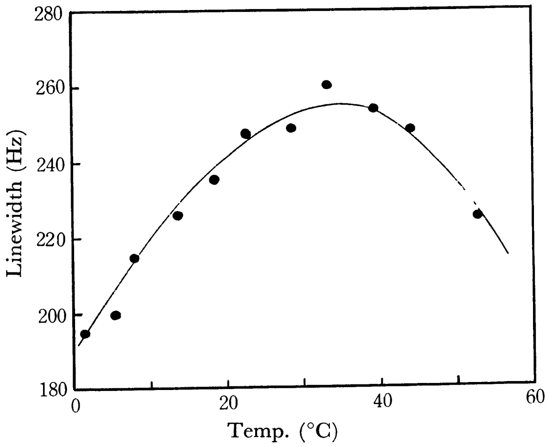

Fig. 3. Temperature dependence of the linewidth for agarose gel containing 79% of water.

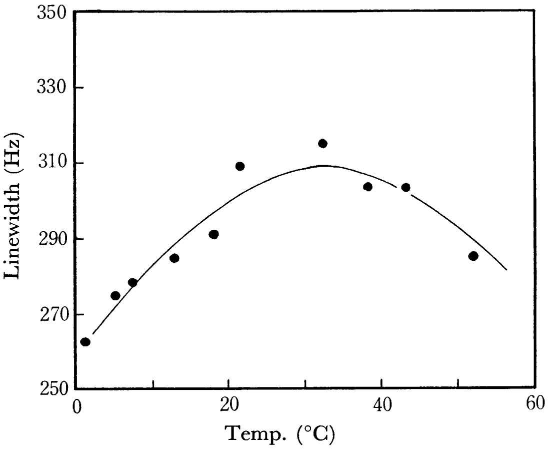

Fig. 4. Temperature dependence of the linewidth for agarose gel containing 72% of water.

The temperature dependence of the line-width for κ-carrageenan gels are illustrated in Fig. 5. For the gel containing 90.5% of water, the linewidth is not so dependent on the temperature as that of agarose gels. The linewidth, however, does not decrease with an increase in the temperature. Therefore, an anomalous temperature dependence was observed in such a

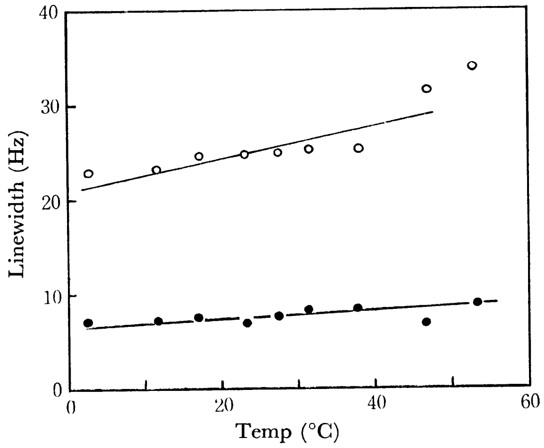

Fig. 5. Temperature effect on the linewidth of κ-carrageenan gel containing 85% of water (-○-) and 90.5% (-●-).

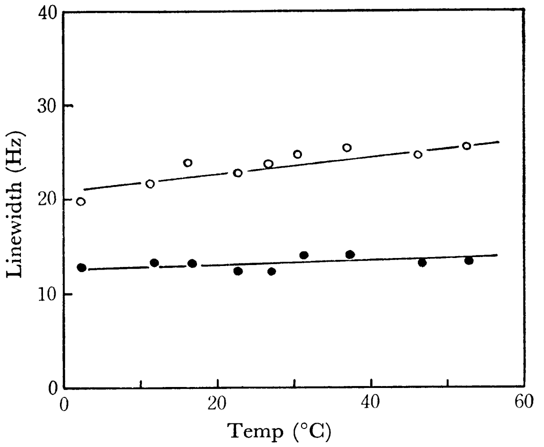

Fig. 6. Temperature effect on the linewidth of curdlan type polysaccharide gels containing 89.5% of water (-○-) and 91.8% (-●-).

gel. The κ-carrageenan gel containing 85% of water exhibited an evident anomalous temperature dependence of the linewidth, but it was, not so marked as in the agarose gels.

Figure 6 presents the temperature dependence of the NMR linewidth of the water proton in curdlan-type polysaccharide gels. The gels containing both 91.8 and 89% of water showed an anomalous temperature dependence similar to that of κ-carrageenan gels.

As was expected, the anomalous temperature dependence was confirmed in the κ-carrageenan gels and curdlan-type polysaccharide gels, though it was not so pronounced as in agarose gels. In addition, the maximum point in the linewidth *vs.* temperature curve, which was observed for agarose gels, was not detected for either κ-carrageenan gels or curdlan-type polysaccharide gels.

(3) λ-Carrageenan Gels. The gel strength of λ-carrgeenan gels is known to be not so strong as that of κ-carrageenan gels. The temperature dependence of the linewidth is postulated for the λ-carrageenan gels containing 93.5 and 88% of water in Fig. 7. These λ-carrageenan gels show a decrease rather than an increase in the linewidth with an elevation of the temperature. The anomalous temperature dependence was not so enormous as that of the other gels.

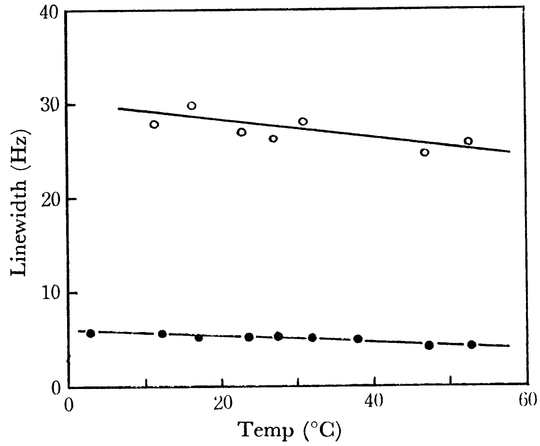

Fig. 7. Temperature effect on the linewidth of λ-carrageenan gel containing 88% of water (-○-) and 93.5% (-●-).

Agarose, κ-carrageenan, curdlan-type polysaccharide, and λ-carrageenan gels exhibited an anomalous temperature dependence of the NMR linewidth of their water proton. The magnitudes of the anomalities were in the order; agarose>κ-carrageenan, curdlan type polysaccharide>λ-carrageenan gels. As was expected, this new finding regarding the anomalous temperature dependence of the line-width is closely related with the gel network. Agarose gels which have a strong network give the most marked anomality.

Discussion

Anomalous temperature effects on the nuclear magnetic resonance linewidth of the water proton in gels were found not only in agarose gels, but also in κ-carrageenan and curdlan-type polysaccharide, λ-carrageenan gels. The effect was the most prominent in the agarose gels.

The linewidth change depends not only on the motional state of the proton, but also on the so-called exchange association, the impurities, and so on. As is shown in Fig. 1, the line-shape of water in gels may be Lorentzian in the upper part and rather Gaussian in the lower part. The line shape remains unchanged in the temperature range surveyed. Proton exchange is estimated to be so rapid as to show no splitting in this system. Therefore, if the exchange association occurs, the linewidth can be expected to be narrowed. The impurities which influence the nuclear magnetic resonance are negligible, as is shown in Table 1. Therefore, the factors other than the motional state of the proton cannot explain those anomalous phenomena.

In view of the motional state of the proton, we assumed that this anomality can be explained by the relationship between the gel strength and the motional state of the water proton. Agarose gels are endowed with a high gel strength and elasticity, though κ-carrageenan and curdlan-type polysaccharide gels are flexible in texture. λ-carrageenan gels have a low gel strength. The magnitude of the anomality decreased with a decrease in the gel strength. The anomalous temperature effect was found to be related with the strength of the gel network.

Gels, in general, are constructed of a macromolecular network consisting of a number of small cavities. Gels can enclose a large amount of water inside these cavities. Such cavities may be called "micro-space." The dimensions of the micro-space are estimated to be in the order from several Å to 10^2 Å. The properties of the micro-space, depend of course, on the steric properties of the network polymer. For instance, water inside the micro-space constructed of a rigid network may be markedly prevented from thermally expanding, because the expansion coefficient of the network polymer is much smaller than that of water, as was shown previously.[3)] In other words, the motional state of water in a gel is closely related with the properties of the micro-space. Therefore, the above anomalous temperature effect on the linewidth of water can be explained in connection with the properties of the micro-space.

The important points regarding the behavior of water in gels are that the lower water contents of agarose gel containing less than 30% show no anomality in their temperature dependence, and that such an anomality is observed only above the transition points in the range from 0 to -20°C, as is shown in Fig. 8. At least three states of water, such as W_1, W_2, and W_3, can be considered for the behavior of water molecules in macromolecular gels, as was proposed previously, where W_1 and W_3 correspond to the so-called free water and bound water respectively.[2,3)] The agarose gels containing less than 30% of water are dominated mainly by the specific state which corresponds to W_3. Therefore, most of the water in such gels may be stuck so tight by hydroxyl groups of agarose that it behaves just like the network polymer, even in thermal expansion. That is why no anomality was observed in such lower-water-content agarose gels.

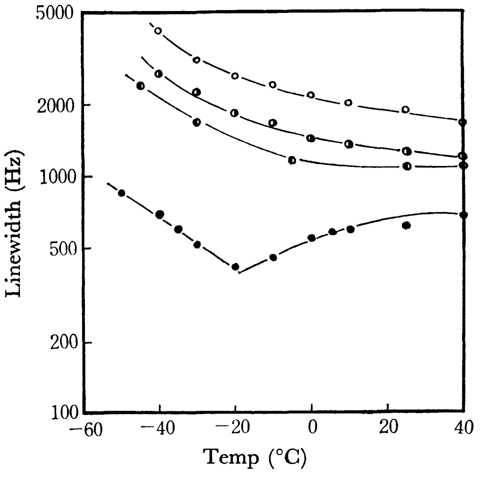

Fig. 8. NMR linewidth changes between -60°C and 40°C for varied water contents of agarose gel. -○- 10%, -◑- 19%, -◐-21.3%, -●- 43%.

Agarose gels containing a large amount of water gave an anomalous temperature dependence of the linewidth. The network polymer of the gel has not only hydrophylic sites, but also hydrophobic sites. Supposed that such hydrophobic sites expel water molecules in the micro-space, water molecules may lose their freedom.

Another important point is that the maxima were seen in the curve of the linewidth against the temperature for agarose gels, though the other gels gave no maxima. Though the gel-sol transition point of agarose gels is approximately 80°C, agarose gels soften around the above maxima. Such a softening is not so obvious in the other polysaccharide gels. In gels, a tremendous difference in thermal expansion coefficient can be supposed between the network polymer and entrapped water, as was indicated previously. The thermal expansion of enclosed water may be supposed on the basis of the network polymer. The thermal expansion of gels should be determined at the balance of the expansions of the network polymer and enclosed water. When the network polymer, however, cannot restrict the expansion of water, in other words, the nodes of the network polymer are relaxed with an increase in the temperature, the thermal expansion coefficient of the gels increases and the enclosed water becomes rather mobile. The maxima in the curve of the linewidth against the temperature for agarose gels can be attributed to such a point. Therefore, agarose gels should soften and the linewidth of water decreases with an increase in the temperature above this point.

On the basis of these results and discussion, the new finding regarding the anomalous temperature dependence can be explained by attributing it to the effect of the micro-space. The restriction of the motional state appears when the extreme difference between the framework of the micro-space, *i.e.*, the network polymer, and the enclosed molecules, *i.e.*, water, is in the physicochemical properties, such as the thermal expansion coefficient.

Brey[7)] calculated the activation energy of water adsorbed on lysozyme through the temperature dependence of the NMR linewidth. As a lysozyme containing 488 mg/g of water showed a negative activation energy, he eliminated its activation energy from the other results without any discussion, though the temperature dependence was persented. These anomalous phenomena can be explained by the micro-space effect.

Summary

An anomalous temperature dependence of the NMR linewidth was found for water in macromolecular gels. In other words, the linewidth of the water proton increased with an elevation in the temperature.

The magnitude of this anomality was in the following order: agarose gels$>\kappa$-carrageenan, curdlan-type polysaccharide gels$>\lambda$-carrageenan gels. The gel with a rigid network gave an extinct anomality compared with the flexible network of the gel. This new-found anomality was explained by the "Micro-Space Effect."

[7)] W. S. Brey, Jr., T. E. Evans, L. H. Hitzrot, *J. Colliod Interface Sci.*, **26**, 306 (1968).