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The Properties of Water in Macromolecular Gels. VI. The Relationship between the Rheological Properties and the States of Water in Macromolecular Gels

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The relationship between the rheological properties and the states of water in macromolecular gels is investigated by both the rheological and nuclear magnetic resonance methods. In accordance with the stress-strain curve, macromolecular gels are shown to be classifiable into: (1) an agarose type of gel which shows great gel strength and brittleness at breaking; (2) a κ-carrageenan type of gel showing great gel strength, but ductility at breaking, and (3) a λ-carrageenan type of gel, which has very little gel strength and is pasty. The dependence of the NMR line-width of water in gels on the polymer concentration and on the temperature reveals that the motional state of water in gels is restricted in this order: the agarose type of gel > the κ-carrageenan type of gel > the λ-carrageenan type of gel. These results show that the state of water is closely related with the rheological properties of macromolecular gels.

The states of water existing in polysaccharide gels, such as agarose-, κ- and λ-carrageenan-, and curdlan-type polysaccharide gels, have previously been studied by the electrochemical method,[1,2] by means of nuclear magnetic reasonance,[3,4] and by the dilatometric method.[5] These studies have revealed that so-called free water is a mixture of at least two different states of water; one is comparable with ordinary water except that it is prevented from flowing out, and the other is less mobile than ordinary water, but more mobile than so-called bound water.

On the other hand, various rheological types of gel can be found in polysaccharide gels. Agarose gels, for instance, exhibit brittleness, while k-carrageenan gels show ductility. It has not been clarified yet, however, what affects these rheological properties of macromolecular gels. This investigation was carried out with the aim of clarifying how to correlate the states of water to the rheological properties of macromolecular gels.

The states of water existing in gels are quite difficult to survey by means of spectroscopic measurements because of their poor transparency. Nuclear magnetic resonance (NMR) methods may be more suitable for such investigations, for the motional states of water can be estimated through the NMR line-width of the proton signal. Agarose-, κ- and λ-carrageenan-, and curdlan-type polysaccharide gels were measured for the NMR line-width of their water, and the line-widths were compared with their rheological properties.

Experimental

Samples. Agarose-, κ-carrageenan-, λ-carrageenan-, and curdlan-type polysaccharide gels prepared at the required water contents were used as the samples. Curdlan-type polysaccharide is a bacterial gel-forming β–1,3-glucan obtained from the culture filtrate of a mutant strain (NTK–U, IFO 13140) of *Alcaligenes faecalis var. myxogenes*, strain 10C3K.[6]

Gel Strength and Elasticity Measurement. The stress-strain curves for gels were obtained at 25 °C by the use of a gel-strength tester. The water contents of gels were measured before and after the mechanical test.

NMR Spectra. The proton NMR spectra were obtained using a JNM–C–60HL high-resolution NMR spectrometer, as has been described previously.[3] The sample was sealed in a standard sample tube (5 mm ϕ O.D.). The temperature was measured by means of a copper constantan thermocouple near the sample tube.

Results

Rheological Properties of Various Polysaccharide Gels. Gel strength is known as a gel property. It is not sufficient, however, as a basis for profiling the rheologi-

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

[2] J. Mizuguchi, M. Takahashi, and M. Aizawa, *ibid.*, **91**, 961 (1970).

[3] M. Aizawa, J. Mizuguchi, S. Suzuki, S. Hayashi, T. Suzuki, N. Mitomo, and H. Toyama, This Bulletin, **45**, 3031 (1972).

[4] M. Aizawa, S. Suzuki, T. Suzuki, and H. Toyama, *ibid.*, (in press).

[5] M. Aizawa and S. Suzuki, *ibid.*, **44**, 2967 (1971).

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

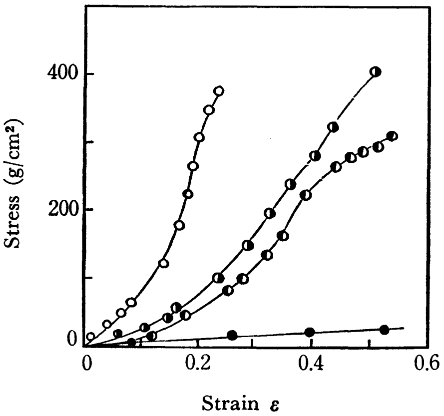

Fig. 1. Stress-strain curves of agarose (—○—), curdlan-type polysaccharide (—◑—), κ-carrangeenan (—◐—) and λ-carrageenan (—●—) gels containing 2% of polysaccharide.

cal properties of gels, because it shows only the maximum at the breaking point. A stress-strain curve may be convenient as a basis for classifying the rheological properties of the various polysaccharide gels.

The stress-strain curves at 25 °C are shown in Fig. 1 for agarose-, κ-carrageenan-, λ-carrageenan-, and curdlan-type polysaccharide gels. The polysaccharide content of each is 2%. The strain is indicated as ε in the abscissa, showing the ratio of the contraction to the length of the gel which is free from stress.

In the range of low stress, the stress correlates linearly with the strain. The gradients of the stress-strain curves, *i. e.*, the elastic moduli, increase in the following order: λ-carrageenan<κ-carrageenan and curdlan-type polysaccharide<agarose. The end points of the curves show the stress at the breaking point, the so-called gel strength. The λ-carrageenan gel has very little gel strength. The other three kinds of gels exhibit comparable gel strengths.

The stress-strain curve of agarose gel has an inflection point at $\varepsilon=0.2$, following the abrupt rise between this inflection point and the breaking point. Thus, this type of gel shows brittleness. κ-Carrageenan gel gives an inflection point in the stress-strain curve around $\varepsilon=0.4$, and the slope of the line in the range from this inflection point to the breaking point is moderate compared to that of the agarose gel. The curdlan-type polysaccharide gel resembles the κ-carrageenan gels in its rheological properties.

On the other hand, the λ-carrageenan gel markedly contracts when subjected to even a slight stress, showing paste-like properties. Therefore, the polysaccharide gels investigated can be classified as follows:

(1) The agarose type of gel, which shows great gel strength and brittleness at breaking.

(2) The κ-carrageenan type of gel, which shows great gel strength and ductility at breaking, and

(3) The λ-carrageenan type of gel, which shows very little gel strength and paste-like properties.

NMR Line-width of Water in Gels. The polysaccharide gels can be classified into three groups according to the rheological properties mentioned above. As the NMR line-width is known to be related to the spin-spin relaxation time, T_2 which indicates the motional states of nuclear spin, and is known to become narrow when the nucleus is in a vigorous motional state,[7] the NMR line-width of water was investigated for these three types of polysaccharide gel.

For agarose-, κ- and λ-carrageenan-, and curdlan-type polysaccharide gels, the NMR line-width of water is illustrated at various concentrations in Fig. 2. The line-width is indicated in Hz units as the half-width of the maximum peak height. The line-width widens with the increase in the polysaccharide concentration for each gel. The concentration dependence of the line-width is most prominent for agarose gels, as is shown in Fig. 2. In contrast, the line-width changes only slightly with the increase in the λ-carrageenan concentration. The concentration dependences of the line-width of water in κ-carrageenan and curdlan-type polysaccharide gels lie between those agarose and λ-carrageenan gels. These results indicate that water is more restricted in the agarose type of gel than in the κ-carrageenan and λ-carrageenan types of gel.

Temperature Dependence of the NMR Line-width of Water in Gels. The temperature dependence of the NMR line-width of water can offer effective information concerning the states of water in gels. The temperature dependence of the line-width below 0 °C is shown in Fig. 3 for agarose, κ-carrageenan, and curdlan-type polysaccharide gels containing 92% of water by weight. The line-width increases with the temperature decrease, this shows the restriction of the motional state of water. Most water existing in gels changes in phase from a liquid into a solid at 0 °C, this accompanies the drastic change of the line-width. As the line-width of water in the solid state, frozen water, is too wide to be detected by a high-resolution NMR spectrometer, the NMR signal of water frozen at 0 °C may be eliminated below 0 °C. Therefore, the NMR line-width shown in Fig. 3 has been determined by studying the water molecules which remains unfrozen even in this temperature range. In Fig. 3, an anomalous temperature dependence of the line-width is found in the temperature range from 0 to −20 °C. This anomalous temperature depen-

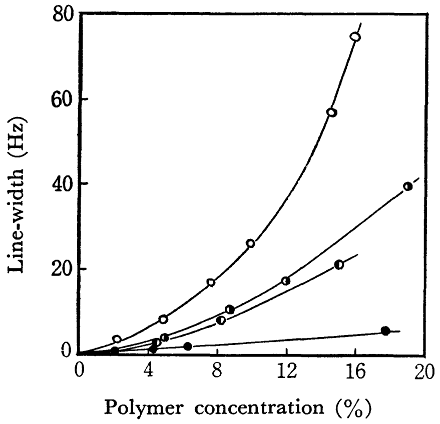

Fig. 2. NMR line-width of water in agarose (—○—), curdlan-type polysaccharide (—◑—), κ-carrageenan (—◐—) and λ-carrageenan (—●—) gels.

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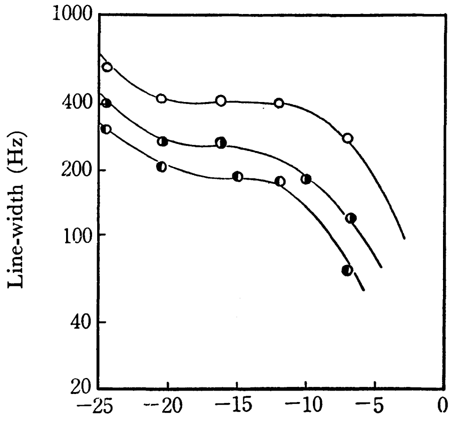

Fig. 3. Temperature dependence of NMR line-width of water in agarose (—○—), curdlan-type polysaccharide (—◑—) and κ-carrageenan (—◐—) gels containing 92% of water.

dence may be caused by the phase-transition of water which remains unfrozen below 0 °C.

Moreover, water in an agarose gel exhibits a wider line-width in this temperature range than the other two kinds of polysaccharide gel. This indicates that the motional state of water which remains unfrozen below 0 °C is restricted more extremely in an agarose gel than in the other two kinds of gel. The restriction of the motional state of water which remains unfrozen below 0 °C is in the order: agarose>curdlan-type polysaccharide>κ-carrageenan gels. This restriction order quite agrees with the results derived from the polymer-concentration dependence of the NMR line-width.

Generally, the NMR line-width gets narrower with an increase in the temperature (motional narrowing). On the contrary, the line-width of water existing in polysaccharide gels was found to become wider with the increase in the temperature above 0 °C, as has been reported previously.[4] Such an anomalous temperature dependence of the line-width can be expected to be closely related to the rheological properties of polysaccharide gels.

The temperature effects on the NMR line-width of water are presented in Fig. 4 for agarose-, κ-carrageenan-, λ-carrageenan-, and curdlan-type polysaccharide gels containing 92, 91, and 92% of water respectively. In Fig. 4, the line-width of water in an agarose gel is shown to increase with the increase in temperature up to approximately 35 °C, and then to decrease above this temperature. The anomalous temperature effect is the most prominent in the agarose gel. Water involved in the curdlan-type polysaccharide and κ-carrageenan gels increases the line-width, with a slight inclination, with the increase in the temperature, giving no maxima. This anomalous effect is not so marked, but is rather, negligible in a λ-carrageenan gel. The magnitude of the anomalous temperature effect on the line-width decreases in this order: agarose>curdlan-type polysaccharide and κ-carrageenan>λ-carrageenan gels.

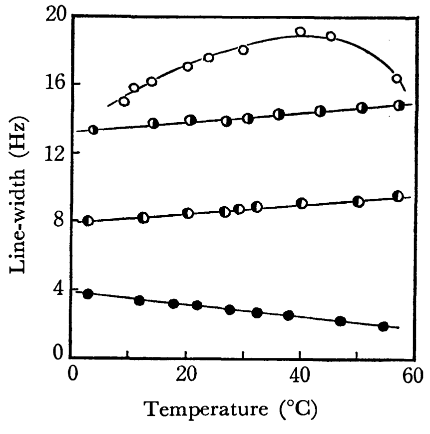

Fig. 4. Anomalous temperature dependence of NMR line-width of water in agarose (—○—), curdlan-type polysaccharide (—◑—), κ-carrageenan (—◐—) and λ-carrageenan (—●—) gels containing 92, 92, 91 and 92% of water respectively.

Discussion

The nuclear magnetic resonance properties of water in polysaccharide gels may be summarized as follows:

(1) For agarose gels, the line-width of water depends markedly on the polysaccharide concentration of the gel, the anomalous temperature effect on the line-width is the most prominent, and the line-width is wider than in the other gels at the same concentration and the same temperature.

(2) The line-width of water in λ-carrageenan gels is not so dependent on the polymer concentration, and the anomalous temperature effect on the line-width is rather negligible.

(3) The properties of the water involved in curdlan-type polysaccharide and κ-carrageenan gels are intermediate between those of the above two types of polysaccharide gels. As was shown in the previous papers,[3,4] these properties reflect the motional state of water in gels. These results indicate that the motional state of water is restricted in this order: agarose>curdlan-type polysaccharide and κ-carrageenan>λ-carrageenan gels.

The above order corresponds reasonably well to that of the rheological classification. In other words, the water involved in the rheologically-strong gels is extremely restricted or immobilized. Therefore, a close relationship between the rheological properties of gels and the states of water existing in it was revealed.

Summary

The nuclear magnetic resonance properties of water showed that the restriction effect on the motional state of water was the most marked in agarose gels among the polysaccharide gels surveyed, and that it was loosened in this order: agarose>curdlan-type polysaccharide, κ-carrageenan>λ-carrageenan gels. On the other hand, the polysaccharide gels were revealed to be classifiable into three groups according to their rheological properties: (1) the agarose type of gel, (2) the κ-carrageenan type of gel, and (3) the λ-carrageenan type of gel. A close relationship between the motional state of water and the rheological properties was found on the basis of the above results.