NMR Study on the Sol-gel Transition of Gellan Gum

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Alkali metal NMR and carbon-13 NMR were carried out to investigate the affinity of counter cation to gellan gum and its gelation mechanism. Cations are involved in long-lived binding to well-defined sites on gellan molecules by non-electrolytic affinity interaction with the order of $Rb^+ > K^+ > Na^+$. These sites may consist of some oxygen functions, which may be similar geometry to crown ethers, in addition to the carboxylic acid groups arranged in such a way to fit the size of ions.

Key Words: Sol-gel Transition, gellan gum, Multinuclear NMR

1. INTRODUCTION

Gellan gum is an extracellular polysaccharide produced by Pseudomonas elodea composed of tetrasaccharide repeating units: 1,3-linked β-D-glucose (1, 3-Glu*p*), 1,4-linked β-D-glucuronic acid (1, 4-Glu*p*A), 1,4-linked β-D-glucose (1, 4-Glu*p*), and 1,4-linked α-L-rhamnose (1, 3-Rha*p*) (Fig. 1).[1,2] It is well known that gellan gum forms a thermoreversible gel. Although the gelation mechanism is not fully elucidated, the formation of ordered structure and subsequent alignment of the ordered region is considered to be responsible for gelation. Ions play an essential role in the molecular processes associated with conformational transition and gelation of gellan gum. Grasdalen and Smidsrød suggested that gel formation of polysaccharides is a two-step process, consisting of, first an intramolecular transition to an ordered conformation and, second, the salt bridges between ordered segments of the chain.[3] It is known that the gelation of gellan gum is markedly enhanced by the presence of group I cation in solution.[4]
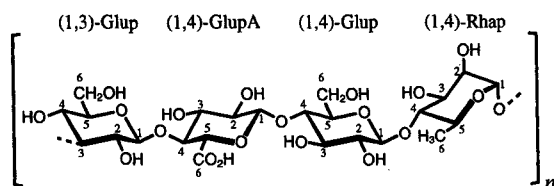
The roles of group I cations in gelation of gellan gum aqueous solution and the motional states of cations in the vicinity of the sol-gel transition are not clarified yet. This article describes the full assignment of the ^{13}C NMR spectra of gellan gum, and the use of such data, coupled with multinuclear NMR, to investigate the interaction between gellan gum and group I cations.

2. EXPERIMENTAL

Sample Preparation: Sodium type Gellan gum was kindly supplied by San-ei Gen FFI Co. (Osaka, Japan) and was used without further purification. NaCl, KCl and RbCl (Wako Pure Chemical Industries Ltd., Osaka) were used as supplied. The powdered gellan was dissolved in salt aqueous solutions at 70°C and then heat the solution up to 90°C to dissolve gellan completely. Solutions were cooled to 25°C and were kept at that temperature for 12h to form gels.

Table I. Sample Characteristics

Sample No.	Gellan gum (wt%)	NaCl (mM)	KCl (mM)	RbCl (mM)	T_m[1]) (°C)
I	1.5	20	–	–	35
II	1.5	–	20	–	40
III	1.5	–	–	20	40

1) T_m is determined by the falling ball method.

Fig.1 Chemical structure of the repeating unit of gellan gum.

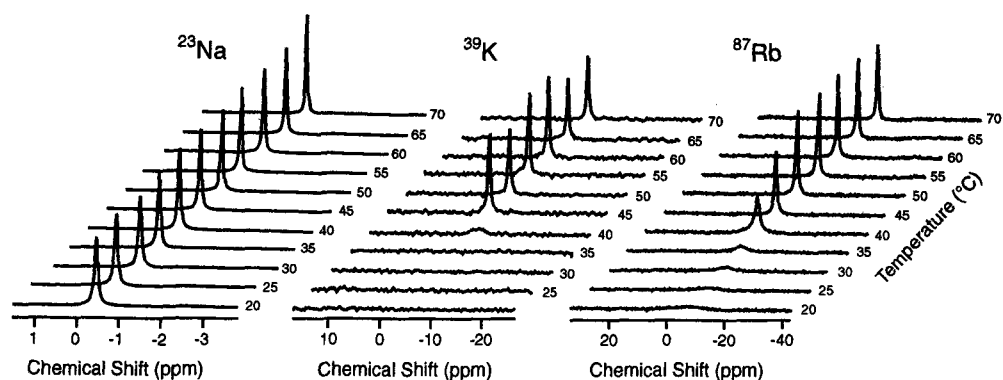**^{1}H and ^{13}C NMR:** The ^{1}H and ^{13}C NMR NMR spectra

Fig.2 Temperature dependence of the spectra of ^{23}Na, ^{39}K and ^{87}Rb.

were obtained with a JEOL JNM-LA600 spectrometer equipped with a 14.09 T superconducting magnet. The probe temperature was changed from 25°C to 80°C with an interval 5.0°C and was kept constant ±0.5°C by the passage of thermostated air during accumulation. All the experiments were performed using Na type gellan gum dissolved in H_2O/D_2O mixed solvent (50/50 in volume) in a 5-mm NMR tube. Chmical shifts were referenced to the TSP signal at 0.00 ppm as an external standard. One-dimensional ^{1}H and ^{13}C NMR NMR spectra were measured with 32K data points and a sweep width of 12005 Hz for ^{1}H, and 40650 Hz for ^{13}C. DQF-COSY experiments were carried out in phase-sensitive mode with spectral width of 3475 Hz in both dimensions and data matrix size 1024 (F_2) x 512 (F_1). PFG (pulse-field gradient)-HMQC spectra were acquired into a 1024 (F_2) x 256 (F_1) data matrix in size by the magnitude mode with 2613 Hz for ^{1}H, and 25389 Hz for ^{13}C. PFG-HMBC experiments were obtained with spectral width of 2713 Hz in the ^{1}H dimension and 25840 Hz in the ^{13}C dimension. The 2D spectral data were acquired in the magnitude mode with 1024 (F_2) x 256 (F_1) data matrix in size and the evolution time to long-range coupling constants was optimized for 8.25 Hz (60 msec). The phase sensitive HSQC-HOHAHA experiments were carried out with spectral width of 3000 Hz for ^{1}H and 8019 Hz for ^{13}C. The 2D spectral data were acquired with 1024 (F_2) x 256 (F_1) points in size and the mixing time was set to 30 40 and 60 msec, respectively. All two-dimensional data sets were zero filled to 2 times data points for t_1 dimension during data processing.

Multinuclear NMR: Multinuclear NMR spectra were obtained with a JEOL JNM-LA400 spectrometer equipped with a 9.39 T superconducting magnet, respectively. The ^{23}Na, ^{39}K and ^{87}Rb resonance frequencies at this field were 106, 18.5 and 131 MHz respectively. The probe temperature was changed from 25°C to 60°C with an interval 5.0°C and was kept constant ±0.5°C by the passage of thermostated air during accumulation. The temperature was measured with a calibrated thermocouple. Chemical shifts were

referenced to the signal obtained from 1 mol/L aqueous salt solutions at 0.00 ppm as an external standard. The spin-spin relaxation time, T_2^*, was calculated from the line-width at half height of the absorption spectrum, $\Delta v_{1/2}$, according to $T_2^* = 1/\pi\Delta v_{1/2}$. The compositions and the melting temperatures of three samples studied here are listed in Table I.

RESULT AND DISCUSSION

Motional State of Group I Cations: The temperature dependence of the NMR spectra of ^{23}Na, ^{39}K and ^{87}Rb in samples I, II and III on the heating process is shown in Fig. 2. The line shape of resonance spectra of ^{39}K and ^{87}Rb sharpens markedly in the vicinity of melting temperature of the gels, while that of ^{23}Na is almost independent of temperature. The sensitivity of cations to its immediate environment is related to the quadrupolar interactions with the local electric field gradient (EFG). As typical for group I cations in aqueous solutions, a single line is observed in Fig.2. At the temperatures above melting point, the cations are fully hydrated and experiences a reasonably symmetric EFG with the result that line widths, Δv, are small and chemical shifts, σ, are almost same as that in aqueous solutions at same temperatures. It is important to note, however, that the line widths at higher temperatures than melting points are still appreciably greater than Δv for aqueous electrolytes at same temperatures. This indicates that cations still sense carrageenan matrix even in the sol-state.

In macromolecular systems, rapid exchange between relatively free and bound sites often occurs, and this situation is described by the expression

$$\Delta v_{obs} = P_b\Delta v_b + P_f\Delta v_f \quad (1)$$

$$\sigma_{obs} = P_b\sigma_b + P_f\sigma_f \quad (2)$$

where the subscripts f and b denote free and bound state, respectively. P signifies the mole fraction in each state and $P_b + P_f = 1$. The use of these expressions in the subsequent analysis gives useful insight into the behavior of group I cations but only in a semi-quantitative manner.

Fig. 3 shows the decomposed spectra of ^{23}Na, ^{39}K and ^{87}Rb in Sample I, II and III at 25°C, melting temperature
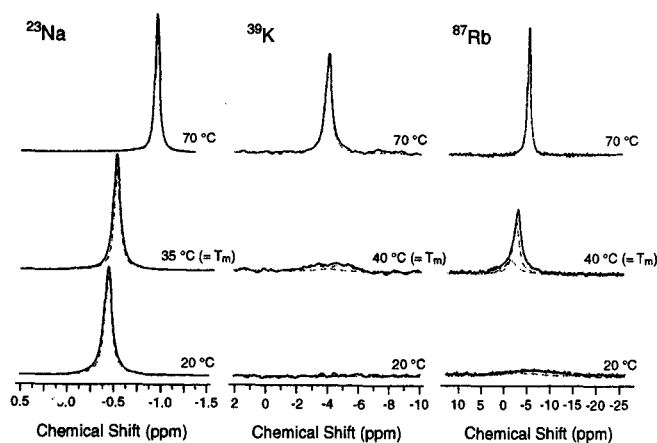
Fig. 3 Decomposed spectra of ^{23}Na, ^{39}K and ^{87}Rb at 20 ¡C, at melting temperature (Tm) and at 70 ¡C. *Broken curves* represent the simulated peaks.
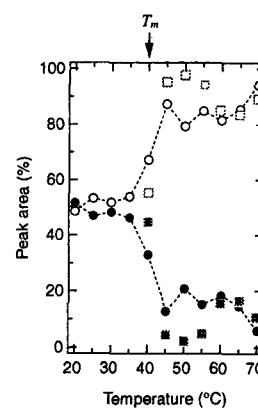

Fig. 4 Temperature dependence of the peak area of ^{39}K (circle) and ^{87}Rb (square) for both short-lived (open symbol) and long-lived binding ions (closed symbol).

and 70°C. The ^{23}Na NMR lines from gellan reveals that the molecular motion of ^{23}Na is isotropic and rapid enough to average the quadrupole interaction to zero. A single line constituting 100 % of the total intensity is observed in the temperature range studied here. At 70°C, the ^{39}K and ^{87}Rb ions NMR lines from gellan gum are also typical of isotropic solutions with rapid molecular motions. Single lines constituting 100 % of the total intensities are observed. On lowering the temperature, a very marked and abrupt change in line shapes take place with gelation, indicating changes in the mode of counter-ion binding (Fig. 4). While the molecular motions are still isotropic and rapid enough to average the quadrupole interaction to zero, non-exponential relaxation of interacting ^{39}K and ^{87}Rb ions apparently produces two superposed signals. These line shapes agree with the numerical calculations for the quadrupole relaxation of spin 3/2 nuclei engaged in two-site exchange, which is studied by Bull et al.[5]   For the long correlation time of the bound ions, $\tau_c \gg 1/2\pi\nu_0$ (ν_0 is the resonance NMR frequency), the transverse relaxation was show to be a sum of the four exponentials with coefficients corresponding to the intensities of the lines found in the quadrupole-split multiplet. The central sharp line in this multiplet, showing 50 % of the total intensity, corresponds to the most slowly decaying component, which is independent of static quadrupole interaction. The other faster relaxing components give rise to much broader lines in the NMR spectrum. Therefore, ^{39}K and ^{87}Rb NMR signals from gellan gum in the gel-state

indicate that the bound ions so slow that the condition $\tau_c \gg 1/2\pi\nu_0$ is fulfilled, and the correlation time, τ_c, is greater than 10^{-8} sec. A motional restriction of at least 3 orders of magnitude for bound ions relative to free ions, for which $\tau_c \approx 10^{-11}$sec, is indicative of long-lived binding of the ^{39}K and ^{87}Rb ions at well-defined binding sites on the gellan, where ions reside for longer than 10^{-8} sec. These results indicate that some of the cations in each sample may be involved in long-lived binding to well-defined sites on the gellan molecules while others are relatively free. The relaxation rate of long-lived binding ion is significantly greater than that of short-lived one. Chemical shifts respond in a similar manner. The states are presumed to be in equilibrium through rapid exchange except in the gel-state. In the gel state cation environment becomes distorted, leading to increases for the long-lived values of both P and σ. Therefore, the dramatic changes in observed values for NMR parameters are due to the fact that in equations (1) and (2), both P and σ are changed.

Full Assignment of Carbon-13 NMR Spectrum of Gellan Gum: ^{1}H and ^{13}C signals for the gellan gum were assigned (Table II) using a combination of phase-sensitive DQF-COSY, HMQC, HMBC and phase-sensitive HSQC HOHAHA experiments.[6]   Phase-sensitive HSQC HOHAHA on gellan gum is shown in Fig. 5.

Carbon-13 NMR Study of the Sol-gel transition of Gellan Gum: In previous section, the roles of salts were investigated to elucidate the gelation mechanism of

Table II. ^{13}C chemical shifts for gellan gum

Residue	Chemical Shift (ppm)					
	1	2	3	4	5	6
(1,3)-Glup	105.77	77.35	85.83	71.66	79.50	64.33
(1,4)-GlupA	105.62	76.28	77.93	84.17	78.90	177.91
(1,4)-Glup	106.49	77.25	77.93	82.40	78.35	63.84
(1,4)-Rhap	104.09	73.93	73.74	84.78	70.88	20.25
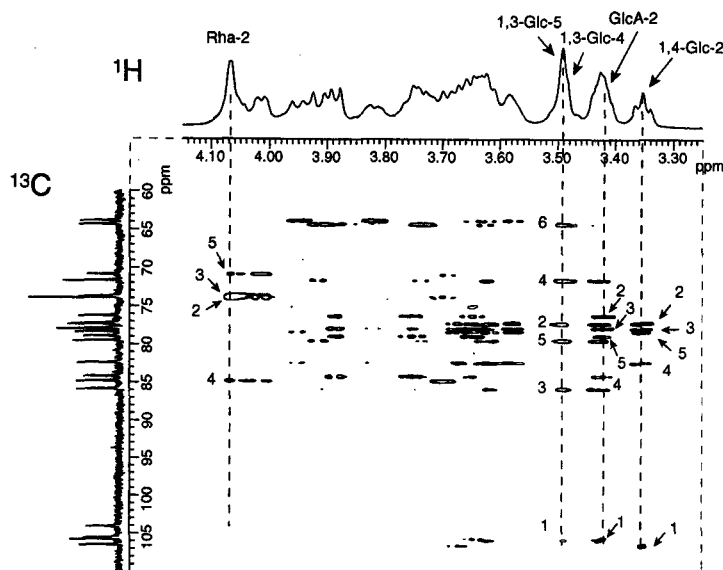
Fig. 5 ^{1}H-^{13}C HSQC-HOHAHA Spectrum of gellan gum in D$_2$O/H$_2$O=50/50 (in volume) at 80$_1$C.

gellan gum by means of alkali metal NMR. X-ray diffraction analysis has revealed the ordered arrays of polysaccharide conformations in the condensed phase. However the conformational properties of polysaccharides in solution and gel phase has not been fully clarified yet. NMR provides a powerful tool for studying these phenomena at molecular level. To avoid the effect of the selectivity of counter cations, we prepared the Na, K and Rb types of gellan. Fig. 6 shows the ^{13}C shifts of (1,3)-Glup-2, (1,4)-GlupA-6, (1,4)-Glup-6 and (1,4)-Rhap-6 of Na, K and Rb types of gellan gum. (1,3)-Glup-2, (1,4)-GlupA-6 and (1,4)-Glup-6 carbons of K and Rb types of gellan exhibit abrupt changes in chemical shift in the vicinity of

melting temperatures, while these carbons of Na type gellan show monotonous change. On the other hand, (1,4)-Rhap-6 carbon does not show any remarkable change in chemical shift with temperature in all types of gellan.

These results indicate that some of the cations in each sample may be involved in well-defined sites on gellan molecules by non-electrolytic affinity interaction with the order of Rb$^+$ > K$^+$ > Na$^+$. These sites may consist of some oxygen functions, which may be similar geometry to crown ethers, in addition to the carboxylic acid groups arranged in such a way to fit the size of ions.

CONCLUSION

Alkali metal NMR and carbon-13 NMR were carried out to investigate the affinity of counter cation to gellan gum and its gelation mechanism. Cations are involved in long-lived binding to well-defined sites on gellan molecules by non-electrolytic affinity interaction with the order of Rb$^+$ > K$^+$ > Na$^+$. These sites may consist of some oxygen functions, which may be similar geometry to crown ethers, in addition to the carboxylic acid groups arranged in such a way to fit the size of ions.

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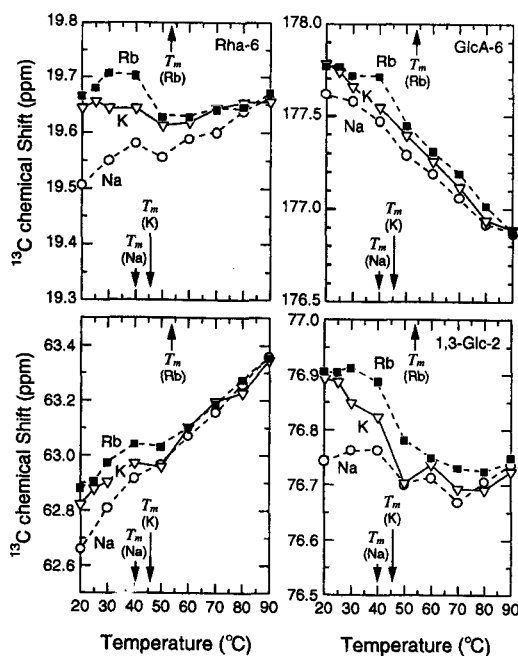[6] A. J. Jay et al. Carbohydr. Polym., **35**,179 (1998).

Fig. 6 ^{13}C chemical shift of Na, K and Rb-type gellan gum as a function of temperature (heating process).