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Interactions of paramagnetic metal ions with gellan gum studied by ESR and NMR methods

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The characterization of the gellan–paramagnetic metal ion complex has been investigated by ESR and NMR methods in aqueous solution of sodium-type gellan. The resonance of the carboxyl carbon in the D-glucuronate unit in ^{13}C-NMR spectra of gellan is selectively broadened with the addition of the Mn(II) ion in sol state. This is due to the paramagnetic interaction between the carbon and the Mn(II) ion. The resonances of other units of gellan are not affected, showing that Mn(II) ions interact with the carboxyl groups in the D-glucuronate unit. Water proton relaxation measurements indicate that water molecules are well coordinated to the gellan–Mn(II) ion complex in gel state. ESR measurements of the Mn(II) ion give the dissociation constant of 3.2×10^{-5} and 6.5×10^{-4} M, and the number of the binding site of 0.12 and 0.33 per repeated unit in sol and gel states, respectively. The latter suggests that in sol state a Mn(II) ion is surrounded by several residues in a randomly coiled gellan polymer and in gel state a Mn(II) ion binds two or three D-glucuronate units on neighboring gellan molecules. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

It has been known that gelation of aqueous solution of gellan gum is promoted in the presence of cations, especially divalent metal cations such as Ca^{2+} and Mg^{2+}, and so on. The gelation temperature is shifted to much higher temperatures, and a pronouncedly stable gel is formed at the higher metal concentrations (Moritaka *et al.*, 1991; Day *et al.*, 1988). It is considered that divalent cations bind to the carboxyl groups in the glucuronic acid unit and form a complex, leading to a stable intermolecular association or a crosslinked structure; however, the detailed mechanism has not been clarified. It is of interest to directly observe the properties of metal ions interacting with gellan. Previously we reported on the interactions of the Mn(II) ion with the potassium-type gellan gel investigated by electron spin resonance (ESR) measurements (Tsutsumi *et al.*, 1993). In this work, to obtain further information on the interactions of divalent cations with gellan, the ESR measurements of the Mn(II) ion in the aqueous sodium-type gellan solution were carried out. The ESR spectra were taken at the various metal ion concentrations in order to see the gellan sol to gel transition caused by the metal ions.

^{13}C high resolution NMR measurements were made to reveal directly the binding site of the Mn(II) ion in the gellan. The spin-lattice relaxation time of the water proton was measured to obtain information on the coordination state of water to the gellan–Mn(II) ion complex.

EXPERIMENTAL

Gellan modified to the sodium (3.3 wt%) type was supplied by the Kelco Division of Merck & Co., Inc. The powdered sample was mixed with distilled water and stirred at 90°C to obtain a homogeneous solution. The concentration was adjusted to 3 mmol/dm^3 and the pH was 6.5. To this solution a small amount of $MnCl_2$ aqueous solution was added. Mn(II) concentration was changed in the range from 0.1 to 4.0 mmol/dm^3. The solution was introduced into a quartz capillary tube with a diameter of 0.3 mm and sealed off for the ESR measurements.

ESR spectra of Mn(II) ions were taken with a JEOL FEX-1XG (Japan Electron Optical Laboratory Co. Ltd), operating at a microwave frequency of 9.24–9.25 GHz and a power of 1 mW, causing no saturation effects. The 100 kHz field modulation width was 6.3 gauss. The signal intensity was defined as the average peak-to-peak height of the derivative six lines of Mn(II) (Tsutsumi *et al.*, 1993) and was expressed in units of the

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spectral peak height of diphenylpicrylhydrazyl (DPPH) as the external reference. ^{1}H- and ^{13}C-NMR measurements were made using a JEOL FX270 spectrometer at the resonance frequencies of 270 and 67.5 MHz, respectively. The spin-lattice relaxation times of the water proton for the $MnCl_2$ aqueous solutions with and without gellan were measured by the inversion recovery method. The relaxation efficiency ε, which gives information on the coordination state of the water to gellan–Mn(II) ion complex, was estimated using the relation (Eisinger *et al.*, 1962):

$$\varepsilon = (1/T^*_{1p})/(1/T_{1p}), \tag{1}$$

$$1/T_{1p} = 1/T_1 - 1/T_{10}, \tag{2}$$

$$1/T^*_{1p} = 1/T^*_1 - 1/T_{10}, \tag{3}$$

where T_{10} and T_1 are spin-lattice relaxation times for the water proton without and with Mn(II) ions, respectively. T^*_1 is the spin lattice relaxation time for the water proton in the presence of the Mn(II) ion and gellan.

RESULTS AND DISCUSSION

Figure 1 shows the ^{13}C-NMR spectra of gellan measured at 60°C in D_2O containing different Mn(II) ion concentrations, *f*, which is defined as the molar ratio of the Mn(II) ion to gellan. Although the detailed signal assignment has not been made at the present stage, the carbonyl peak (175.2 ppm) for glucuronic acid ring, methylol peaks (61.2 and 61.7 ppm) for glucose rings, and the methyl peak (17.6 ppm) for the rhamnose ring are clearly seen. The carbonyl peak shows marked broadening and a decrease in intensity at $f = 9.7 \times 10^{-4}$. This peak completely disappears at $f = 4.3 \times 10^{-3}$. These observations strongly suggest that the Mn(II) ion selectively interacts with the carboxyl group, and the effects of the paramagnetic dipolar appear markedly. For other peaks, no effects of Mn(II) are observed except for the peak at 76.1 ppm, where paramagnetic broadening occurs. This peak is assigned as C_5 in the glucuronic acid ring, since it directly attaches to the carboxyl carbon with which the Mn(II) ion interacts.

Figure 2 shows the plots of the ESR signal intensities at 30°C against the total Mn(II) ion concentration, $[M]_t$, for the aqueous Mn(II) solutions without and with polymers, which are denoted as I_t and I_f, respectively. It has been reported that the ESR signal of Mn(II) disappears when it binds to a macromolecule (Tsutsumi *et al.*, 1986, 1993; Hikichi *et al.*, 1984; Reed & Cohn, 1970). It is seen that $[M]_f$ is considerably reduced, indicating that a certain amount of Mn(II) ions was bound to gellan gum. The molar concentration of free, $[M]_f$, and bound, $[M]_b$, Mn(II) ions were estimated from I_t and I_f using the relationships:

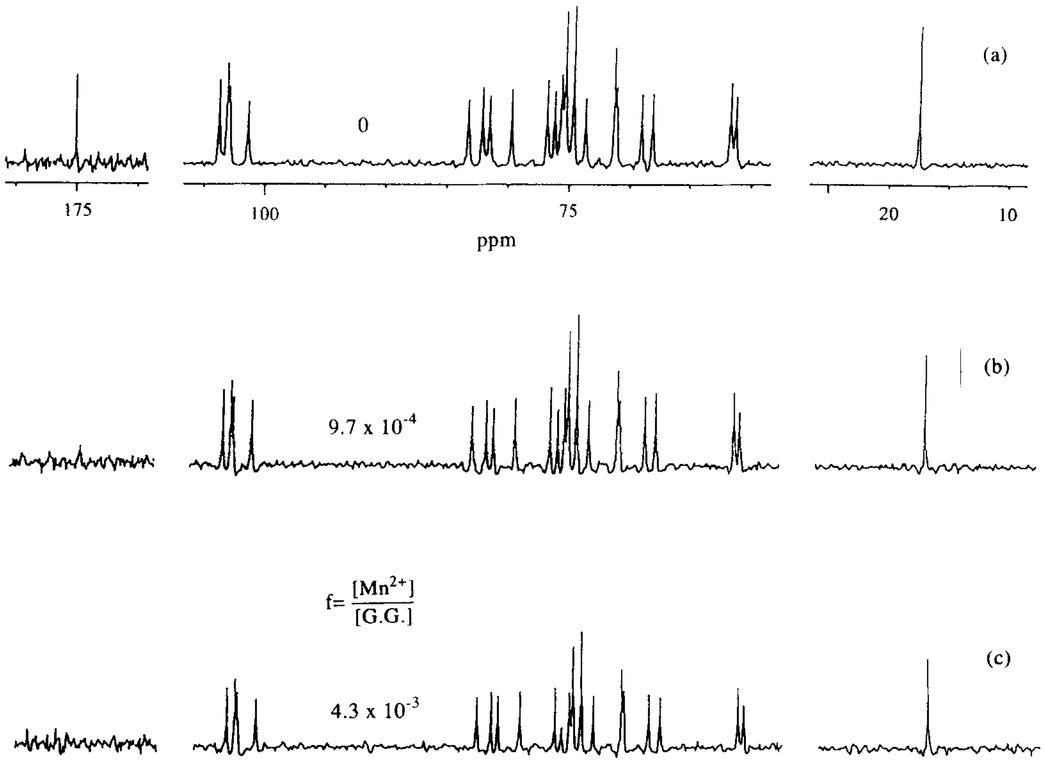

Fig. 1. ^{13}C-NMR spectra of sodium-type gellan gum in D_2O at 60°C. Polymer concentration is 0.015 mol/dm^3. Chemical shift is referred to tetramethylsilane. Concentrations of Mn(II) ion, *f*, expressed in mol ratio to polymer are 0.0 (a), 9.7×10^{-4} (b), and 4.3×10^{-3} (c).

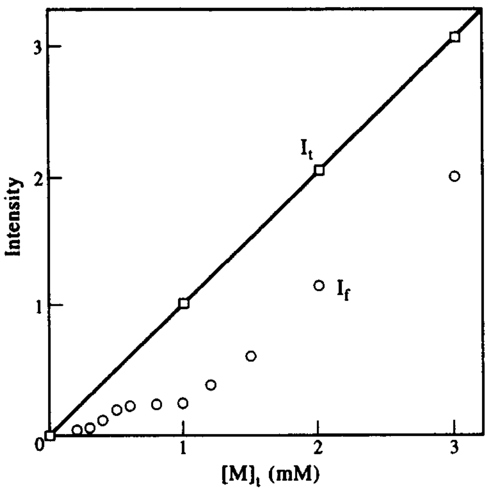

Fig. 2. ESR signal intensity of Mn(II) versus $[M]_t$ for aqueous solutions with (○ I_f, 0.003 mol/dm^3) and without (□ I_t) gellan gum at 30°C.

$$[M]_f = [M]_t(I_f/I_t) \text{and} \quad (4)$$

$$[M]_b = [M]_t - [M]_f. \quad (5)$$

Figure 3 shows $[M]_f$ and $[M]_b$ plotted against $[M]_t$. $[M]_f$ increases by way of three steps. In the first step, $[M]_t$ =0.0–0.6 mM, and in the third step, $[M]_t$ = 1.0∼3.0 mM, $[M]_f$ increases monotonically; whereas in the seconnd step, $[M]_t$ =0.6–1.0 mM, $[M]_f$ maintains a nearly constant value. These tendencies are observed over the whole temperature range measured in this work, 5–95°C. Solutions are thin at the first step and slightly viscous at the second step. At the third step, the solution is in the gel state. These observations suggest, therefore, that the $[M]_f$–$[M]_t$ (and $[M]_b$–$[M]_t$) curve reflects different states of solutions. In the first step, where ^{13}C-NMR spectra give well-resolved sharp lines, Mn(II) ions interact selectively with carboxyl groups of the randomly coiled gellan gum polymers. In the second step, Mn(II) ions may interact with polymer chains taking different conformations such as the random coil and the double helix and in different molecular association states. This step corresponds, therefore, to the intermediate state between the random coil polymer solution and the crosslinked gel state. It is considered that the rapid increase of $[M]_b$ at this step reflects the increase of Mn(II) ions forming the complex with double helical polymers. In the third step, Mn(II) ions bind only with the double helical gellan where $[M]_b$ keeps nearly a constant value.

In Fig. 4, the relaxation efficiency, ε, obtained from ^{1}H-NMR measurements of water is plotted against $[M]_t$. ε shows characteristic behavior at the three steps observed in ESR measurements. At the first step, $[M]_t$ < 0.6 mmol/dm^3, ε has a small and nearly constant value of 0.7. At the second step, $0.6 < [M]_t < 1.0$ mmol/dm^3, a rapid increase occurs. At the third step, 1.0 mmol/dm^3 < $[M]_t$, ε shows a relatively higher value of 1.6–2.1. Under the condition that the water molecules undergo chemical exchange between the bound and free states with an exchange rate faster than a relaxation rate in the free state, the contribution of the Mn(II) ion to the spin-lattice relaxation time of water molecule T_{1p} in the absence of gellan gum is expressed as (Swift & Connick, 1962; Woesner & Zimmerman, 1963; Luz & Meiboom, 1964):

$$1/T_{1p} = fq/(T_{1M} + \tau_m), \quad (6)$$

where T_{1M} is the spin-lattice relaxation time of the water bound to Mn(II), τ_m the lifetime of the bound water, f the fractional concentration of Mn(II), and q the coordination number of Mn(II) to water. The correlation time of water, τ_c, was estimated to be of the order of 10^{-11}–10^{-12} s from the spin-lattice relaxation time T_{10} and, therefore, the relation $\omega\tau_c \ll 1$ holds, where

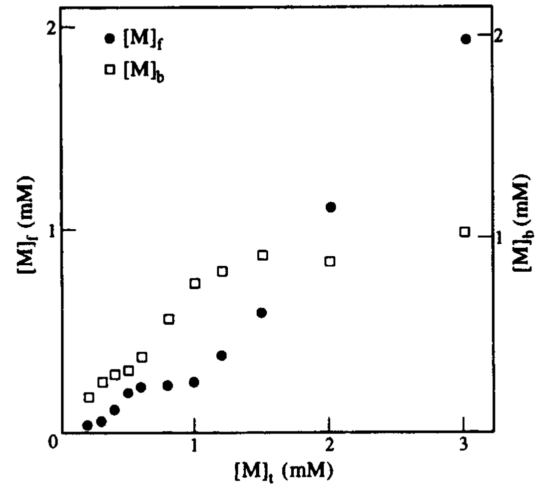

Fig. 3. Plot of $[M]_f$ and $[M]_b$ versus $[M]_t$ for aqueous Mn(II) solution with 0.003 mol/dm^3 gellan gum.

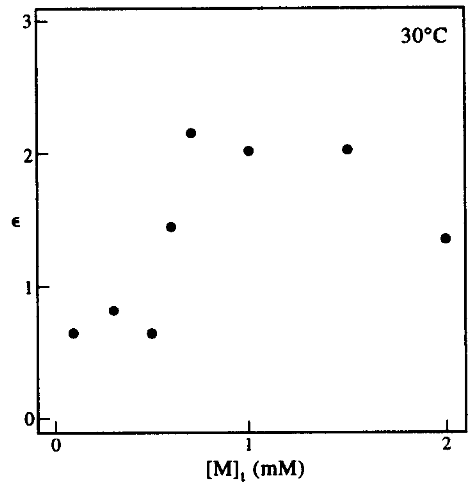

Fig. 4. Plot of the relaxation efficiency factor ε for water proton versus $[M]_t$ at 30°C. Polymer concentration is 0.003 mol/dm^3.

ω is the resonance frequency. In this case, T_{1M} is inversely proportional to τ_c (Solomon, 1955) and an increasing function of temperature, since τ_c is given by the Arrhenius-type temperature dependence. On the other hand, τ_m decreases with increasing temperature. The experimental observation was that T_{1p} increases with increasing temperature indicating that the contribution of T_{1M} is larger than that of τ_m. This may hold for T^*_{1p}, the contribution of Mn(II) to the spin-lattice relaxation time in the presence of gellan gum, since T^*_{1p} also increases with increasing temperature. Thus, in the experimental conditions used, the relationship (6) is rewritten as:

$$\varepsilon = (1/T^*_{1p})/(1/T_{1p}) = (f^*q^*/fq)(\tau^*_c/\tau_c), \quad (7)$$

where f^*, q^* and τ^*_c are the concentration of bound Mn(II) ion, the coordination number of Mn(II) to water, and the correlation time of bound water in the presence of gellan gum, respectively. Here, we can put $f^*/f = [M]_b/[M]_t$. At the first step of $[M]_t$, ESR results show that f^*/f changes between 0.6 and 0.8, and the number of binding sites of Mn(II) per residue n shows a very smaller value of 0.1 (see below), indicating that the Mn(II) ion is trapped within a randomly coiled gellan polymer and the coordination of water is considerably reduced. Therefore, it is suggested that the value of q^* is at most 1 and q^*/q is estimated as 0.17, since q of Mn(II) in water is 6. The value of 0.6–0.8 for ε means that the ratio τ^*_c/τ_c is $\sim$6. In the third step, ESR results showed that f^*/f is reduced to the value 0.3–0.7. Therefore, the higher value of ε 1.5–2.0 at this step comes from the increase of q^*/q or τ^*_c/τ_c due to the gelation. However, as described below, at this step, a Mn(II) ion interacts with 2–3 carboxyl groups, indicating that q^*/q is 0.3–0.5. Under these conditions, we obtain $\tau^*_c/\tau_c \sim 10$, which is not much larger than that obtained in the first step. The latter fact may suggest that even in the gel state, the local motion is rather rapid as for the segmental motion in solution.

The dissociation constant K_d for the Mn(II)–gellan complex and the number of binding sites per residue n can be graphically estimated (Hughes & Klotz, 1956; Jouve *et al.*, 1975) using the relationship:

$$[P]/[M]_b = (K_d/n)(1/[M]_f) + 1/n, \quad (8)$$

where $[P]$ is the total concentration of the polymer. Figures 5 and 6 show plots of $[P]/[M]_b$ against $1/[M]_f$ for the first and third steps at 30°C, respectively. The slope and intercept give K_d =0.032 mM/dm^3 and n =0.12, and K_d =0.065 mM/dm^3 and n =0.33 for the first and third steps, respectively. These results indicate that at the first step a Mn(II) ion is surrounded (trapped) by several (many) residues of a gellan polymer in randomly coiled conformation, since the inverse value of n corresponds to the number of residues (carbonyl groups) which interact with a Mn (II).

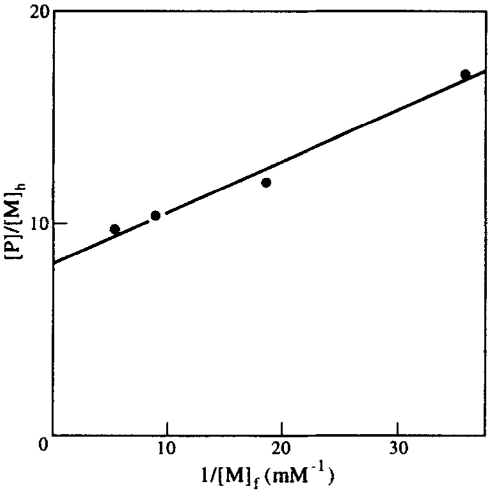

Fig. 5. Plot of $[P]/[M]_b$ versus $1/[M]_f$ for Mn(II)–gellan gum complex in aqueous solution at $[P]$ =0.003 mol/dm^3 and $[M]_t$ =0.0–0.6 mmol/dm^3 (first step) at 30°C.

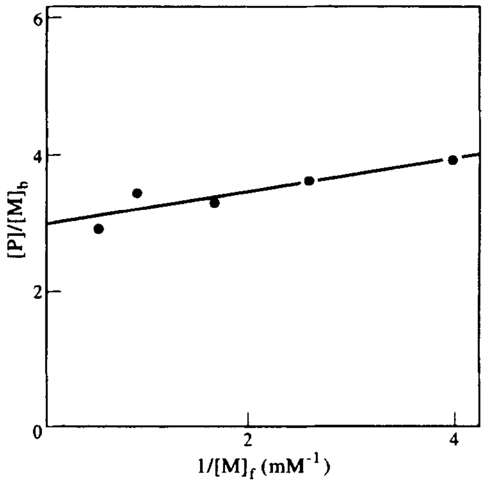

Fig. 6. Plot of $[P]/[M]_b$ versus $1/[M]_f$ for Mn(II)–gellan gum complex in aqueous gel at $[P]$ =0.003 mol/dm^3 and $[M]_t$ = 1.0–3.0 mmol/dm^3 (third step) at 30°C.

In the third step, however, a Mn(II) ion binds to two or three carboxyl groups on the residues in different gellan gum polymers which are associated or cross-linked each other, since the inverse value of n is $\approx$3. It is noted that K_d is considerably smaller than that of the potassium-type gellan gum and other polysaccharides (Tsutsumi *et al.*, 1993), which means that the Mn(II) ion binds relatively strongly to the sodium-type gellan.

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