Macromolecules **1996,** *29,* 5178−5182

Conformational Transition of Polysaccharide Sodium-Gellan Gum in Aqueous Solutions

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Received March 1, 1996; Revised Manuscript Received May 1, 1996[⊗]

ABSTRACT: Temperature dependences of the conformational properties of sodium-type gellan gum in aqueous solutions were studied by osmometry. The measurements were carried out in the range from 25 to 45 °C for aqueous solutions of three sodium chloride concentrations (C_s = 25, 50, and 75 mmol dm^{-3}). It was found that the M_n values obtained at 45, 40, 36, 28, and 25 °C with different C_s agreed with each other and the average M_n values above 36 °C were almost half the values obtained at 28 and 25 °C, suggesting association of two molecules. The values of A_2 obtained at 45, 40, 36, 28, and 25 °C were almost the same at each C_s, while these A_2 values decreased with increasing C_s. The M_n value obtained in solution with C_s = 25 mmol dm^{-3} at 32 °C was slightly higher than those obtained above 36 °C but lower than those obtained below 28 °C, and in solutions with C_s = 50 and 75 mmol dm^{-3}, M_n values could not be determined due to abnormality of the π/c vs c plots. This suggests that a unimer should be in simultaneous equilibrium with a dimer. The data obtained at 32 °C were analyzed by employing a simple unimer/dimer equilibrium model. The equilibrium constant K increases with increasing C_s, which means that the extent of association increases and the proportion of dimer also increases with increasing C_s.

Introduction

Microbial polysaccharide gellam gum, produced by *Pseudomonas elodea*, a is linear anionic polymer consisting of [(3)-β-D-glucose-(1,4)-β-D-glucuronic acid-(1,4)-β-D-glucose (1,4)-α-L-rhamnose-(1)] repeating unit (Figure 1)[1,2] and a carboxyl group in glucuronic acid is responsible for gelation. This polysaccharide is widely used in the food industry and in biotechnology because it forms transparent and heat- or acid-resistant gels.[3]

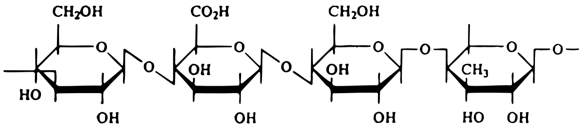

Figure 1. Repeating units of gellan gum molecule.

Table 1. Metal Contents in the Gellan Gum and Na-Gellan Gum Samples[a]

	content/μg g^{-1}			
sample	Na	K	Ca	Mg
gellan gum	4300	59900	8000	1600
Na-gellan gum	36800	300	300	30

[a] Metal contents were measured by flame spectrophotometry (Na, K) and flame atomic absorption spectrometry (Ca, Mg) (Perkin Elmer Model 3100).

The gelation mechanism of gellan gum solutions has been the subject of controversy, but it is now accepted that gellan gum shows a thermoreversible conformational transition from a disordered state (single coil) at high temperature to an ordered state (probably double helix) at low temperature, and subsequent alignment of the ordered regions are responsible for the gel formation.[4−11] The conformational transition temperature of gellan gum has been reported to be around 30 °C.[4−10] Its detailed mechanism, however, has not been clarified yet.

We have studied the coil−helix conformational transition of tetramethylammonium-type gellan gums (TMA-gellan) in aqueous solutions by osmometry.[12−17] From the ionic strength dependences of the number-average molecular weights M_n and the second virial coefficients A_2 of TMA-gellan at 28 and 40 °C, it was found that two molecules should be associated at 28 °C and the ionic strength dependence of A_2 can be quantitatively described by the theory for a charged long-rod model, while at 40 °C that of A_2 seems to be similar to the behavior of linear flexible polyelectrolytes.[13] In this work, temperature dependences of the number-average molecular weights and the second virial coefficients for sodium-type gellan gums (Na-gellans) in dilute solutions were measured by osmometry. The measurements were carried out in the range from 45 to 25 °C for aqueous solutions of three sodium chloride concentrations (C_s = 25, 50, and 75 mmol dm^{-3}).

To obviate any confusion in the subsequent description, we will use superscripts, for example (*28*), to designate the temperature at which the data were obtained and brackets will designate the average of the data, e.g., $M_n^{(28)}$ is the M_n obtained at 28 °C in each C_s solution and $[M_n]^{(28)}$ the average value of $M_n^{(28)}$. For the associated materials, we will call an unassociated molecule a unimer and an associated molecule consisting two unimers a dimer.

Experimental Section

The samples of Na-gellan were prepared from deacetylated gellan gum, kindly supplied by San-Ei Gen F.F.I., Inc. Osaka, Japan (Lot 62058A), by passing through a column of cation exchange resin (Amberlite IR120B) at 60 °C.[12,13,17] The conversion to Na salts was checked by measuring the ionic contents of the Na-gellan samples (Table 1). The Na-gellan was dissolved in aqueous NaCl and stirred for 2 h at 60 °C. The Na-gellan solutions were dialyzed 3−5 days at 45 °C against aqueous NaCl and diluted with this dialyzing solvent. The concentrations of the solutions were determined using a Union-Giken differential refractometer, Model RM-102. A Hewlett-Packard high-speed membrane osmometer, Model 503, having a special type of glass tube was employed for the measurements.[12] To avoid contamination of the solutions with metallic ions, the stainless steel cell in the original design was replaced by a 6-Nylon cell, and other metallic parts of the instrument such as the solvent reservoir and stopcocks were replaced with parts made of glass or synthetic resin.[12]

[⊗] Abstract published in *Advance ACS Abstracts,* June 15, 1996.

S0024-9297(96)00337-3 CCC: $12.00

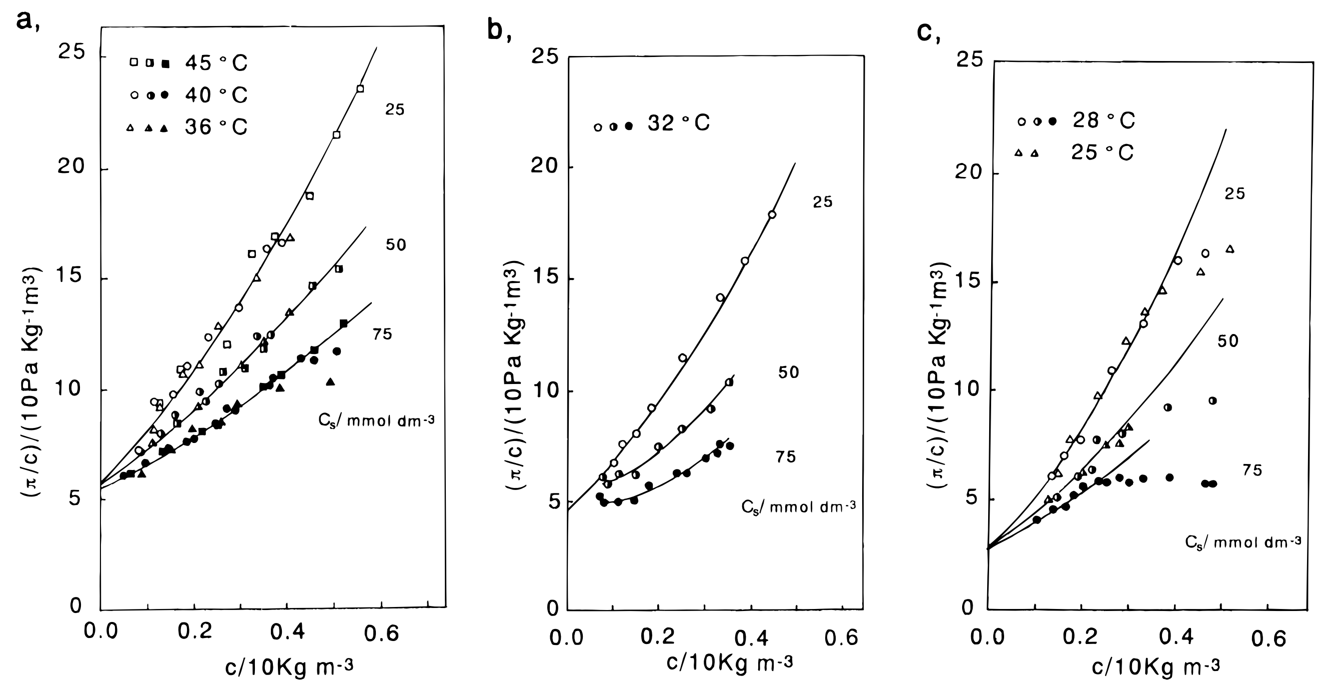

Figure 2. (a) Plots of π/c vs c for NaCl solutions of Na-gellan at 45, 40, and 36 °C. The solid lines denote the values calculated from eq 2 using the values of M_n and A_2 at 40 °C shown in Table 2. (b) Plots of π/c vs c for NaCl solutions of Na-gellan at 32 °C. The solid line is the values calculated from eq 2 using the values of M_n and A_2 at 32 °C shown in Table 2 (25 mmol dm^{-3}) and an empirical fit to the data (50 and 75 mmol dm^{-3}). (c) Plots of π/c vs c for NaCl solutions of Na-gellan at 28 and 25 °C. The solid lines denote the values calculated from eq 2 using the values of M_n and A_2 at 28 °C shown in Table 2.

Results and Discussion

Temperature Dependences of Number-Average Molecular Weights. The osmotic pressure measurements were carried out over the temperature range from 25 to 45 °C for the three aqueous solutions of different NaCl concentrations (C_s = 25, 50, and 75 mmol dm^{-3}). The π/c vs c plots are shown in Figure 2. π is the osmotic pressure and c the polymer concentration. The π/c values almost linearly increased at 45, 40, and 36 °C (except for the solution of C_s = 75 mmol dm^{-3} at 36 °C) in the observed concentration region (Figure 2a). While at 36 (the solution of C_s = 75 mmol dm^{-3}), 28, and 25 °C, π/c values deviated downward above c = ~0.25–0.4 (10 kg m^{-3}) and the lowest polymer concentrations at which these deviations were observed decreased with increasing NaCl concentrations or with decreasing temperature (Figure 2a,c). In these solutions, we noticed a little increase in the solution viscosities at the same temperature and concentration regions. Moreover, in the case of the solution of C_s = 75 mmol dm^{-3} at 25 °C measurements could not be done due to high solution viscosities. It is supposed that interchain aggregations, which should be responsible for gel formation, may occur at least partly. During the measurements, however, the solution was stable as a whole without gelation and good reproducibility of the data was obtained. Therefore, below 28 °C (and the solution of C_s = 75 mmol dm^{-3} at 36 °C) the data in the low-concentration region below c = 0.25–0.4 (10 kg m^{-3}) were used for the following calculations.

It is well-known that the osmotic pressure of the polymer solution is expressed by the following equation with appropriate values of g.[18]

$$\pi/c = (RT/M_n)[1 + A_2M_nc + g(A_2M_n)^2c^2] \qquad (1)$$

Here R is the gas constant and T the absolute temperature. By assuming $g = {}^1/_4$, eq 1 is rewritten as the following square-root equation.

$$(\pi/c)^{1/2} = (RT/M_n)^{1/2}(1 + M_nA_2c/2) \qquad (2)$$

As shown in Figure 3, the observed values, except for two data (C_s = 50 and 75 mmol dm^{-3} obtained at 32 °C), agree well with this equation, as shown by the straight lines in these figures. Thus, number-average molecular weights M_n and second virial coefficients A_2 were determined from these plots and are listed in Table 2. It was found that at the five temperatures used, 25, 28, 36, 40, and 45 °C, the M_n values at three different C_s values agree each other within the experimental error. The average values of $[M_n]^{(25)}$ and $[M_n]^{(28)}$ are almost twice the values of $[M_n]^{(36)}$, $[M_n]^{(40)}$, and $[M_n]^{(45)}$, which suggest an association of two molecules below 28 °C. In the previous study of the osmometry of TMA-gellan in aqueous (TMA)Cl solutions, we also reported that the M_n value ($9.8_3 \times 10^4$) obtained at 28 °C was almost twice the value ($5.0_1 \times 10^4$) at 40 °C.[13] At 32 °C, the $M_n^{(32)}$ value ($5.5_8 \times 10^4$) obtained for the solution at C_s = 25 (mmol dm^{-3}) is slightly higher than those obtained above 36 °C but lower than those below 28 °C. Moreover, as shown in Figure 2b, the plots of π/c vs c for the solutions with C_s = 50 and 75 mmol dm^{-3} showed upward curvatures below c = ~0.2 (10 kg m^{-3}), and M_n and A_2 values could not be determined due to nonlinearities of the $(\pi/c)^{1/2}$ vs c plots (Figure 3b). These results suggest that unassociated molecules (unimer) may be in simultanious equilibrium with associated molecules (probably dimer) in these solutions at 32 °C.

Temperature and Ionic Strength Dependences of Second Virial Coefficients. The slope of each (π/ c) c plot in Figure 2 gives an A_2 value containing the contribution from the third virial term. The parameter g in eq 1 relates to the third virial coefficient A_3, $g = A_3/A_2{}^2M$. To diminish the third virial contribution, $g = {}^1/_4$ (eq 2) is often used. However, this g value is a rather

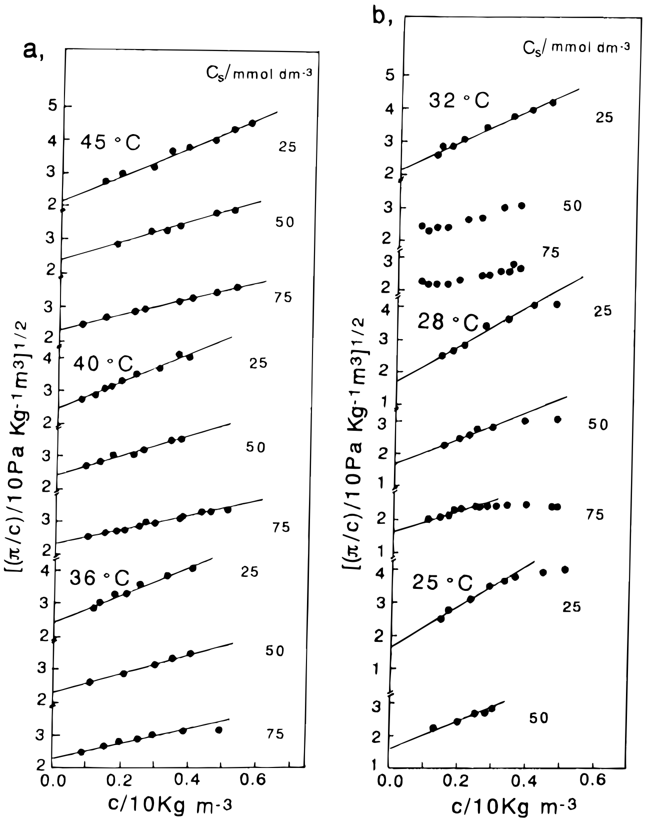

Figure 3. (a) Plots of $(\pi/c)^{1/2}$ vs c for the Na-gellan solutions at 45, 40, and 36 °C. (b) Plots of $(\pi/c)^{1/2}$ vs c for the Na-gellan solutions at 32, 28, and 25 °C.

empirical[19] and its applicability to polyelectrolyte aqueous solutions is not fully established yet. To evaluate A_2 and g separately, we adopted the Stockmayer–Casassa plot,[20] i.e., $[(\pi/cRT) - 1/M_n]/c$ vs c, by using the average M_n value at each temperature obtained above. The plots for each solution fit a single line, and A_2 and g were determined from the intercept and the slope of the line, respectively. The results are also shown in Table 2 together with the data from eq 2. The A_2 values evaluated from these two plots agree well, and the g values obtained by the Stockmayer–Casassa procedure are close to $^1/_4$ as assumed in the above square-root plots. From these results it is considered that eq 1, with $g = {}^1/_4$, holds for the Na-gellan solutions. We showed previously that the g values obtained at 28 and 40 °C from the osmometry of TMA-gellan and Na-gellan in aqueous salt solution over a wide salt concentration range using the Stockmayer–Casassa procedure were also close to $^1/_4$.[13,15]

On the assumption that the macroion is a point particle having $-Z$ charge, the second virial coefficients of ideal solutions can be expressed by eq 3 derived from the theory of Donnan and Guggenheim.[21,22] Here, Z is

$$A_2 = 10^3Z^2/4M^2C_s \qquad (3)$$

the charge of each macroion and C_s the concentration of the salt solution. In the derivation of eq 3, the excluded volume of the polyelectrolytes and electrostatic interactions are tacitly ignored. It was reported that for rigid molecules such as globular protein the contribution of the Donnan effect to the second virial coefficient is usually much greater than the excluded-volume effect except for low charges or high salt concentrations, so the second virial coefficient should be proportional to Z^2 or $1/C_s$.[23–26] For flexible polyelectrolytes the situation is complicated. The size of the polyelectrolyte chain varies with salt concentration due to the electrostatic interactions. It has been considered that the flexible polyelectrolytes have more or less coiled conformation at high ionic strengths. As the ionic strength is decreased, the polyelectrolyte coil is expanded due to the electrostatic repulsion, which can be regarded as a kind of excluded-volume effect. Consequently, the polyelectrolyte chain takes a highly extended form at low ionic strength. A further complication arises that ions, the counterions for instance, tend to be condensed onto the charged macroion and this effect should lead to a reduction of the effective charge on the macroion and a decrease of the apparent second virial coefficients. Obviously, all the above effects are interwined and contribute to the observable phenomena.[24]

We showed that the values of $M_n^{(25)}$ and $M_n^{(28)}$ were almost twice the values of $M_n^{(36)}$, $M_n^{(40)}$, and $M_n^{(45)}$, which suggests the association of two molecules at 25 and 28 °C. Therefore, a conformational change of Na-gellan molecules may occur at the temperature region between 28 and 36 °C. Several authors reported that the molecular conformation of gellan gum changed from a double helix (rodlike form) to a flexible coil with increasing temperature[4–11] and the transition temperature was ~30 °C.[4–10] It is considered that the dimer–unimer transition observed in this study corresponds to the double helix–flexible coil conformational transition.

As shown in Table 2, the values of $A_2^{(25)}$, $A_2^{(28)}$, $A_2^{(36)}$, $A_2^{(40)}$, and $A_2^{(45)}$ were almost the same at each C_s, while the A_2 values decreased with increasing C_s. This result may be interpreted as follows. Even if the helix–coil conformational transition of Na-gellan occurred with increasing temperature, the flexible coil chain is highly extended and takes more or less a rodlike form in the C_s solutions due to electrostatic repulsions. Therefore, the observed A_2 values remain almost unchanged over the temperature range of 25–45 °C, and they could be described qualitatively by eq 3. The experimental A_2 values are considerably lower than those calculated from eq 3; the ratio of the observed A_2 to the calculated ones were ~0.35, 0.5, and 0.55 at C_s = 25, 50, and 75 mmol dm^{-3}, respectively. These observations should be interpreted in terms of the phenomenon of counterion condensation by electrostatic interactions. Previously, we determined A_2 values of TMA-gellan in aqueous (TMA)Cl solutions of different C_s ranging from 12.5 to 500 (mmol dm^{-3}) at 28 and 40 °C and showed that, below C_s = ~75 mmol dm^{-3}, the A_2 values at the two temperatures were coincident and proportional to $1/C_s$ but lower than the calculated ones; above C_s = ~75 mmol dm^{-3}, the A_2 values at 40 °C were smaller than those at 28 °C.[13]

Concentration Dependences of Apparent Molecular Weights. For nonassociating polymers where M_n is concentration independent, it is possible to obtain the true molecular weight from a plot of π/c vs c using eq 1. For associating polymers, however, where M_n is concentration dependent, only the apparent molecular weight M_{napp} can be obtained. It has been shown that a simple unimer/dimer equilibrium model, discussed in detail by Elias,[27] describes well the change in molecular weight with concentration in dilute solutions. This model assumes that the unimer M_I with number-

Table 2. Number-Average Molecular Weights and Second Virial Coefficients for the Na-Gellan in NaCl Solutions

	$M_n \times 10^{-4}$ [a]					
C_s/mmol dm^{-3}	25 °C	28 °C	32 °C	36 °C	40 °C	45 °C
25	9.6_0	9.2_8	5.5_8	4.3_0	4.4_6	4.1_1
50	9.4_4	9.1_7		4.4_0	4.4_6	4.3_9
75		9.6_1		4.7_0	4.9_1	5.0_0
⟨average⟩	⟨9.5_2⟩	⟨9.3_5⟩		⟨4.4_7⟩	⟨4.6_1⟩	⟨4.5_0⟩

	$A_2 \times 10^3$/mol kg^{-2} m^3											
	25 °C		28 °C		32 °C		36 °C		40 °C		45 °C	
C_s/mmol dm^{-3}	(R)[a]	(SC)	(R)[a]	(SC)	(R)[a]	(SC)	(R)[a]	(SC)	(R)[a]	(SC)	(R)[a]	(SC)
25	8.0_0	8.1	7.8_2	7.2	7.8_0	8.4	7.9_7	8.2	8.0_9	8.7	8.2_1	8.6
50	5.6_0		5.4_9	5.8			5.6_0	5.3	5.7_2	5.6	5.7_4	5.3
75			4.1_4	4.4			4.1_0	3.8	4.0_7	3.4	4.0_4	3.5

	g[b]					
C_s/mmol dm^{-3}	25 °C	28 °C	32 °C	36 °C	40 °C	45 °C
25	0.24	0.34	0.21	0.24	0.19	0.18
50		0.18		0.32	0.27	0.28
75		0.27		0.26	0.36	0.42

[a] Determined by the square-root plots (eq 2). [b] Determined by the Stockmayer–Casassa procedure.

average molecular weight M_{nI} is in an equilibrium with the dimer M_{II}.

$$M_I + M_I \rightleftharpoons M_{II} \tag{4}$$

where $[M_I]$ and $[M_{II}]$ are the mole concentrations of M_I and M_{II}, respectively. The total mole concentration of the unimers contained in solution at polymer concentration c (10 kg m^{-3}) is given as

$$[M_I]_{Total} = c/M_{nI} \tag{5}$$

or

$$[M_I]_{Total} = [M_I] + 2[M_{II}] \tag{6}$$

The apparent number-average molecular weight M_{napp} at polymer concentration c (10 kg m^{-3}) can be written as

$$M_{napp} = c/\{[M_I] + [M_{II}]\} \tag{7}$$

The osmotic pressure of dilute solutions of polymers with molecular weight M_{napp} at concentration c can be expressed by analogy with eq 1.

$$(\pi/c) = (RT/M_{napp})[1 + A_2M_{napp}c + {}^1/_4(A_2M_{napp})^2c^2] \tag{8}$$

Equilibrium constants K for the association defined eq 4 can be written as

$$K = [M_{II}]/[M_I]^2 \quad (\mathrm{m^3\ mol^{-1}}) \tag{9}$$

Equation 9 may be useful for the calculation of Gibbs free energy (ΔG) of the association. Then the ΔG of association such as the formation of dimer is given by

$$\Delta G = -RT \ln K \tag{10}$$

From eqs 4–7, by assuming an appropriate K value in eq 9, we calculated M_{napp} at concentration c using the experimental value of $[M_n]^{(40)} = 4.6 \times 10^4$ as M_{nI} and then the π/c value was evaluated from eq 8 by using this calculated M_{napp} values. In this calculation, we used the experimental A_2 values such as 8.0×10^{-3}, 5.7×10^{-3}, and 4.1×10^{-3} (mol kg^{-2} m^3) for the solutions of C_s = 25, 50, and 75 (mmol dm^{-3}), respectively. The calculated π/c values (indicated by solid lines in Figure 4) are seen to fit reasonably to the observed data where K = 30, 65, and 280 m^3 mol^{-1} for C_s = 25, 50, and 75 mmol dm^{-3}, respectively. The concentration dependences of the apparent molecular weight M_{napp} and of the ratio of $[M_I]/[M_I]_{Total}$ using the same K values are also shown in Figures 5 and 6, respectively.

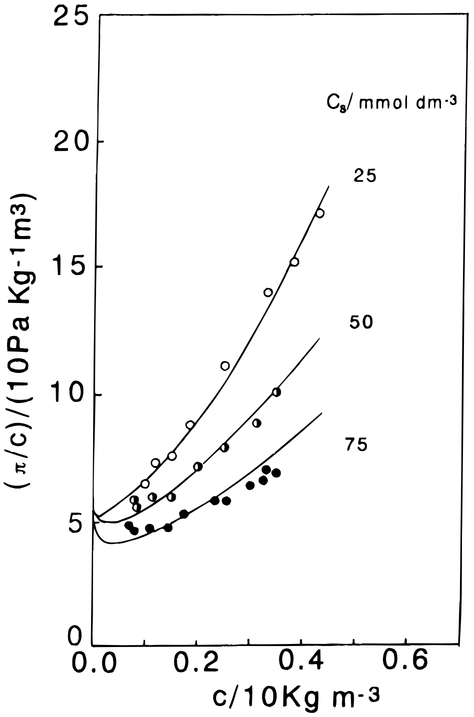

Figure 4. Plots of (π/c) vs c for the Na-gellan at 32 °C in NaCl solutions. The solid lines are calculated from eq 8 using K = 30, 65, and 280 m^3 mol^{-1} for the solutions of C_s = 25, 50, and 75 mmol dm^{-3}, respectively. Details are given in the text.

As shown in Figures 4–6, the equilibrium constant K increases with increasing salt concentration C_s, suggesting that the extent of association increases and the proportion of dimer is higher in high-C_s solutions than in low-C_s solutions. The values of ΔG of the dimer formation at 32 °C calculated using eq 10 are negative and, thus, favorable to the dimer formation process; the values of ΔG calculated are −19, −21, and −24 kJ mol^{-1}

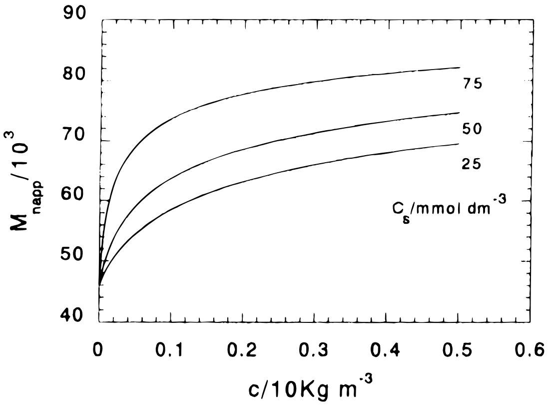

Figure 5. Concentration dependences of the apparent molecular weight $M_{\rm napp}$ of Na-gellan in NaCl solutions calculated from eqs 4–7 and 9. The K values of 30, 65, and 280 m^3 mol^{-1} are used for solutions of C_s = 25, 50, and 75 mmol dm^{-3}, respectively. Details are given in the text.

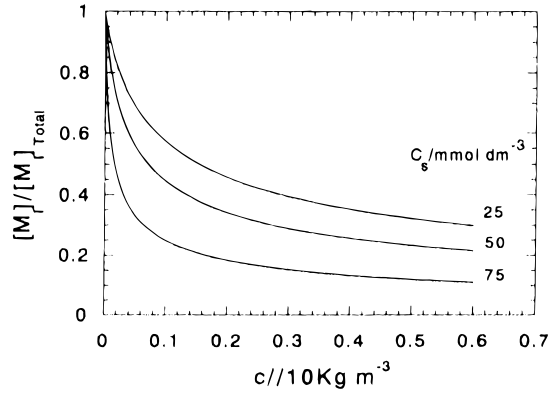

Figure 6. Concentration dependences of the ratio of $[M_I]/[M_I]_{\rm Total}$ in NaCl solutions calculated from eqs 4–7 and 9. The K values of 30, 65, and 280 m^3 mol^{-1} are used for the solutions of C_s = 25, 50, and 75 mmol dm^{-3}, respectively. Details are given in the text.

for the solutions of C_s = 25, 50, and 75 mmol dm^{-3}, respectively. These results could be interpreted that the association of two molecules (the unimer to dimer transition) is promoted with increasing cation concentrations because of screening of electrostatic repulsions between ionized carboxylate groups due to the phenomenon of counterion condensation (ion binding). As to the mechanism of the gelation of gellan gum in solution, it is commonly recognized that the single coil–helix (probably double helix) transition occurs, and aggregation of the helices lead to formation of a junction zone of gels.[4–11] The unimer to dimer transition observed in this study should be attributed to the coil–helix conformational transition prior to gelation. Therefore, it is considered that the electrostatic repulsive interactions between carboxyl groups in gellan molecules are shielded by the increased cations, and as a result, the unimer to dimer transition (probably the coil–helix conformational transition) is promoted with increasing salt concentrations.

Conclusion

The temperature dependences of M_n and A_2 of Na-gellan in aqueous NaCl solutions were measured by osmometry. It was found that the $[M_n]^{(45)}$, $[M_n]^{(40)}$, and $[M_n]^{(36)}$ values were almost half the values of $[M_n]^{(28)}$ and $[M_n]^{(25)}$, which could be interpreted as association of the two molecules below 28 °C in NaCl solutions. The values of $A_2^{(45)}$, $A_2^{(40)}$, $A_2^{(36)}$, $A_2^{(28)}$, and $A_2^{(25)}$ were almost the same at each C_s, while these A_2 values decreased with increasing C_s. At 32 °C, unassociated molecules (unimers) seem to be in simultaneous equilibrium with associated molecules (dimers). The data obtained at 32 °C were analyzed by employing a simple unimer/dimer equilibrium model. It was found that the equilibrium constant K increases with increasing C_s, which means that the extent of association increases and proportion of dimer also increases.

Acknowledgment. The author thanks Prof. K. Ogino of the Chiba Institute of Technology for his discussions and valuable advice for this study. The author also thanks Dr. T. Tanaka of the Science University of Tokyo for the measurements of the contents of metals in the gellan gum sample.

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