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THERMODYNAMICS AND STRUCTURAL ASPECT OF THE GELLING PROCESS IN THE GELLAN GUM/METAL SALT AQUEOUS SOLUTIONS

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Abstract

Gellan gum undergoes gelation by forming domains composed of associated double helices. Here the further random aggregation of associated double helices seems necessary for the formation of the network in the case of Na-gellan with adding NaCl. Gellan gum aqueous solutions were prepared with or without adding various concentrations of NaCl, and their gel-sol transitions were observed by the differential scanning calorimetry (DSC) and the small-angle X-ray scattering (SAXS). The DSC endothermic peaks are attributed to the dissociation of the ordered domains, and the network dissociation in the case of gel samples. The SAXS results are analyzed in terms of the molecular models of associated double helices.

Keywords: crosslinking domain, differential scanning calorimetry, gelation, gellan gum, small-angle X-ray scattering

Introduction

Gellan gum is an extracellular polysaccharide produced by *Pseudomonas elodea*, and is composed of tetrasaccharide repeat units: 1,3-β-*D*-glucose, 1,4-β-*D*-glucuronic acid, 1,4-β-*D*-glucose and 1,4-α-*L*-rhamnose [1] (Fig. 1). Its aqueous solution is known to undergo thermoreversible gelation by cooling in the presence of various metal salts [2]. In the previous paper [3–5], we have shown the crosslinking domain structure composed of associated double helices from the results of small-angle X-ray scattering (SAXS), where the molecular model of the domain is constructed on the basis of the crystallographic data for oriented fibers drawn from gellan gum gel

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[6]. The gelation is thus thought to take place by the formation of double helices and their subsequent alignment, as proposed earlier [7].

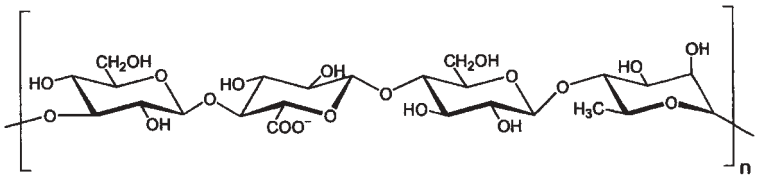

Fig. 1 Repeat units of gellan gum

The gelling characteristics depend on the type and concentration of adding metal salts [8, 9], because gellan gum possesses a carboxylic group in every four repeat units. In this study the Na salt effect on the gelling behavior of gellan gum was investigated by the combination of highly sensitive differential scanning calorimetry (DSC) and small-angle X-ray scattering (SAXS). The highly sensitive DSC is available for the rigorous measurement for the conformational transition in very dilute concentration region. The Na salt requires much higher concentration of gellan to form gel, and its gel has a quite different appearance from the gellan gel form with K or Cs salt.

Experimental

Sample preparation

The Kelco Division of Merck & Co. (Calif., USA) kindly prepared the common gellan gum sample to be shared by the Working Party organized in the Gel Research Group of the Society of the Polymer Science and Technology, Japan. The metal content of the original sample was analyzed to be Na 2.59, K 0.009, Ca 0.029 and Mg 0.001%. The gellan gum was dissolved in water and then mixed with an appropriate amount of NaCl aqueous solution by adjusting the polymer and salt concentrations simultaneously.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) measurements were performed with a DASM-4 scanning microcalorimeter developed by Privalov and Potekhin [11]. The sample solution at 80°C was injected into the DSC cell. After cooling, the scanning was started by heating at a scan rate of 0.5°C min^{-1}.

Small-angle X-ray scattering

The SAXS measurements were performed at BL-10C of the Photon Factory, Tsukuba, Japan. An incident X-ray beam from synchrotron radiation was monochromatized to 1.49 Å with a double-crystal monochromato, and focused to the position of the detector with a bent focusing mirror. The scattered X-ray was detected by a one-dimensional position-sensitive proportional counter positioned approximately

1 m from the sample holder. The sample was contained in a flat glass cell of a path length 0.2 cm and quartz windows of thickness 20 μm. The cell temperature was controlled by circulating water of a constant temperature through the cell holder. The solution was injected into the cell kept at the fixed temperature and left for several tens of minutes prior to the SAXS measurements.

The SAXS intensities were accumulated for 600 s in order to assure sufficient statistical accuracy without degrading the gellan gum by X-ray irradiation. The scattered intensities were corrected with respect to the variation of the incident X-ray flux by monitoring an ion chamber installed in front of the cell holder and the X-ray adsorption of the solution by measuring the incident and transmitted X-ray intensity. The excess scattering intensities were calculated by subtracting the scattering intensities of solvent from those of the gellan gum solution.

Results and discussion

Figure 2 shows the DSC profiles for the variation of the concentration of gellan gum from 1 to 10 mg mL^{-1} without adding salt. Here the heating rate is fixed at 0.5°C min^{-1}. The peak position and shape were consistent regardless of the heating rate changed in the range from 1 to 0.12°C min^{-1}. On cooling process, the same transition temperature and enthalpy variation were obtained as on heating. Therefore the result indicates that we are observing the equilibrium states at each temperature. In the concentration range from 1 to 10 mg mL^{-1}, the solution viscosity increased by cooling, but no gelation took place even at 10°C despite of the definite endothermic peaks being observed. When the gellan concentration increased, the position of peak shifted to higher temperature, and their peak area increased.

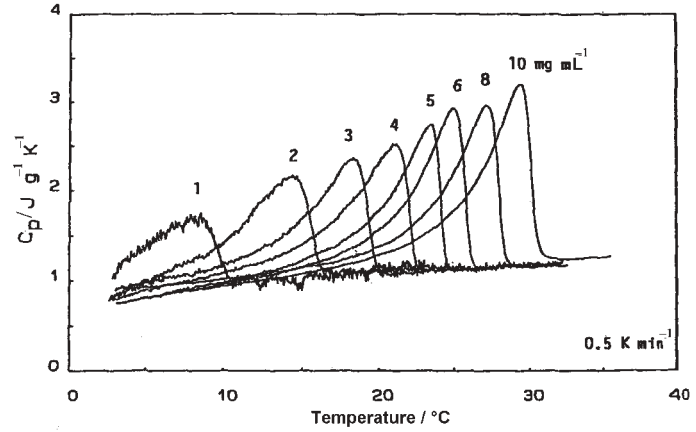

Fig. 2 The DSC curves for the variation of the concentration of gellan gum from 1 to 10 mg mL^{-1} without adding salt

The concentration dependence of gellan gum DSC profiles in 10 mM NaCl aqueous solutions are shown in Fig. 3. Gellan is polyelectrolyte because of carboxylic

groups contained every 4-sugar residue. In consequence, the gelling behavior and/or the domain structure depend much on the type of added metal salt and its concentration. Such a polyelectrolytic effect appeared in the DSC profiles as the shift of the peak position to a higher temperature with increasing the polymer concentration or the salt concentration. That is, the double helical structure of gellan becomes thermodynamically more stable by adding metal salts. As seen from Figs 2 and 3, the DSC profiles are asymmetric, indicating the highly cooperative dissociation process of the double-stranded helical chains of gellan.

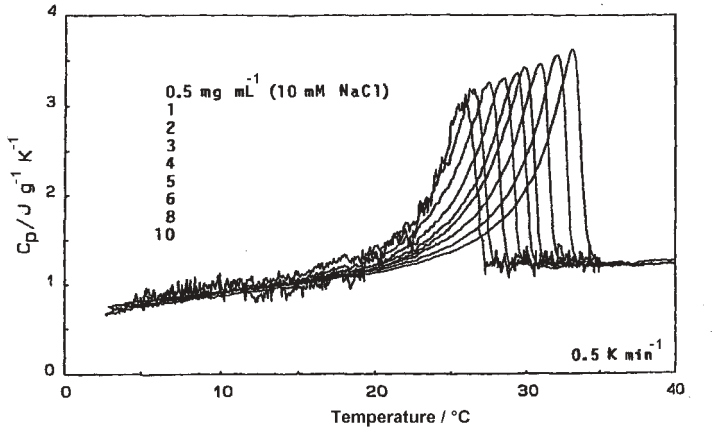

Fig. 3 The DSC curves for the variation of the concentration of gellan gum from 0.5 to 10 mg mL^{-1} in 10 mM NaCl solutions

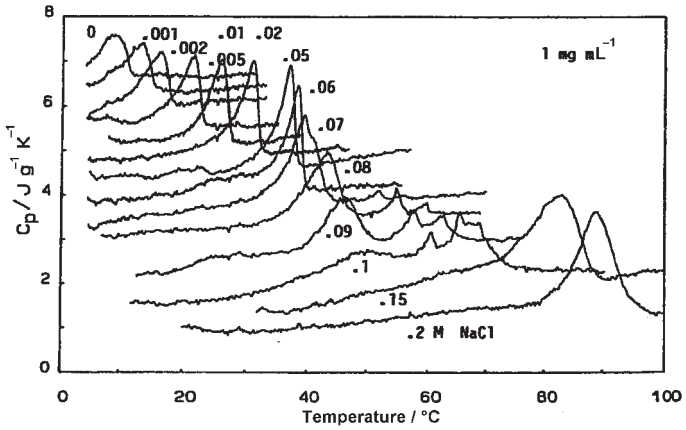

Fig. 4 The salt concentration dependence of DSC curves for 1 mg mL^{-1} gellan gum solutions

The salt effect on the DSC profile is examined for 1 mg mL^{-1} gellan aqueous solutions with added NaCl with the salt concentration being varied from 0 to 0.2 M (Fig. 4).

As the peak shifts to a higher temperature by adding more salt, the DSC profile becomes sharper at first and then splits into multiple peaks when the salt concentration exceeds 60 mM. Gel is formed with the salt concentration over 60 mM NaCl, but its gel is still weak. Gel becomes harder and strong by adding more salt than 150 mM NaCl, where the DSC profile exhibits a single broad peak. This situation becomes much clearer in Fig. 5 when the gellan concentration is increased to 5 mg mL^{-1}, and the multiple peaks were observed for the salt concentration over 60 mM.

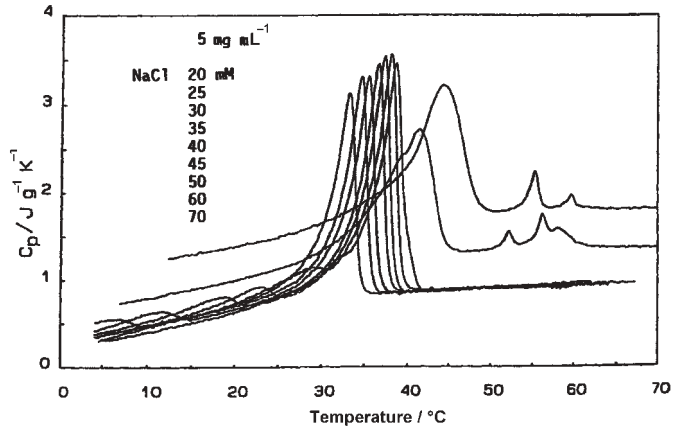

Fig. 5 The salt concentration dependence of DSC curves for 5 mg mL^{-1} gellan gum solutions

The asymmetric DSC profiles observed for gellan with added NaCl less than 60 mM are attributed to the dissociation of associated double helices to single coils. The temperature shift of the peak positions may suggest the polymer and/or salt concentration dependence of the size of the domain composed of associated gellan double helices. That is, the extent of the intermolecular interaction depends on the transition temperature as

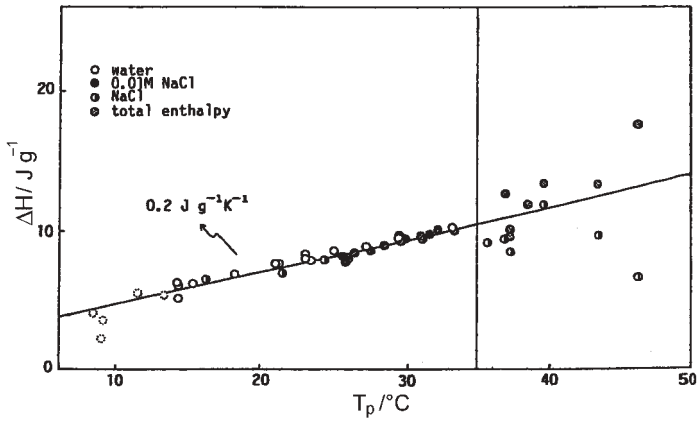

Fig. 6 The relationship between the transition temperature T_p and its enthalpy ΔH

confirmed by the linear relationship between the transition temperature T_p and its enthalpy Δh evaluated from the peak position and area, respectively (Fig. 6). Here the domains composed of associated double helices are rather isolated, and the DSC profiles reflect the thermodynamic behavior of individual domains.

When the salt concentration exceeds 60 mM, the domains grow further and radiate enough branches to tie with other domains. Consequently gelation takes place, and the DSC profiles become broader and exhibit multiple peaks. Here the ordered domains are not anymore isolated but tied to form at first the larger domains composed of randomly connected ordered domains. The larger domains will be characterized as the random aggregates of ordered domains, and serve as crosslinking points responsible for the macroscopic network formation.

The structural details of the domains described above can be elucidated from the small-angle X-ray scattering (SAXS) from the gellan aqueous solutions undergoing sol–gel transition. In present study we observed SAXS under the same conditions as DSC measurements to understand the thermodynamic characteristics from the viewpoint of the change of domain structure. The molecular model of associated gellan gum double helices was proposed from the X-ray diffraction results [6] as shown in Fig. 7. A single helix model was constructed by removing one chain from a double

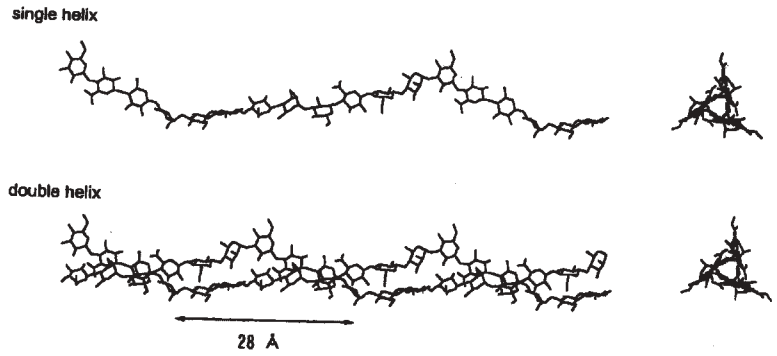

Fig. 7 Gellan gum double helix and single helix models

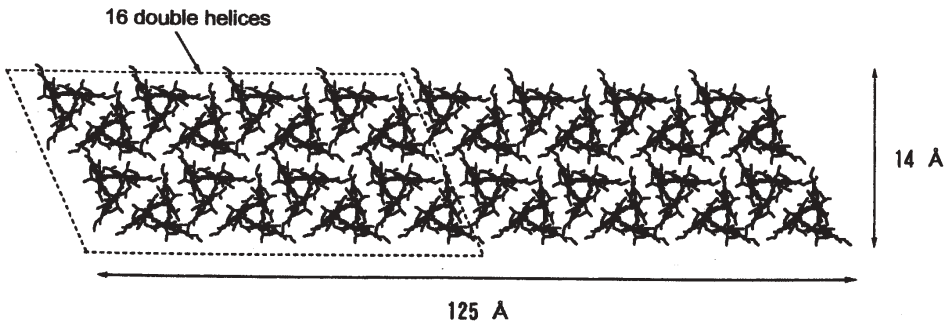

Fig. 8 Molecular model of associated gellan gum chains

helix. The association of double helices takes place by lateral alignment as shown in Fig. 8. The scattering profiles, which are represented by $I(q)$ plotted *vs.* q where $I(q)$ and q denote the scattered intensity and the magnitude of scattering vector given by $(4\pi/\lambda)\sin(\theta/2)$ with θ and λ being the scattering angle and the wavelength of an incident X-ray, are calculated from the atomic coordinates of those associates according to the Debye formula [10]

$$I(q)=\sum_{i=1}^{n} f_i^2 g_i^2(q)+2\sum\sum f_i f_j g_i(q) g_j(q) \frac{\sin(d_{ij}q)}{d_{ij}q} \quad (1)$$

where f_i and d_{ij} denote the atomic scattering factor of the ith atom and the distance between the ith and jth atoms, respectively. The form factor $g_i(q)$ of a single atom is assumed to be represented by the form factor of a sphere possessing the radius R_i equivalent to the van der Waals radius of the ith atom as

$$g_i(q)=\frac{3(\sin(R_i q)-R_i q\cos(R_i q))}{(R_i q)^3} \quad (2)$$

The corresponding scattering profiles are shown in Fig. 9 for a single helix, a double helix, the domain composed of laterally associated 16 double helices, and the domain composed of laterally associated 32 double helices. The observed SAXS profiles exhibit marked temperature dependence as shown by the Kratky plots in Fig. 10 for gellan (5 mg mL^{-1}) in 35 and 70 mM NaCl aqueous solution. Here the temperature was decreased from 70 to 10°C, and gelation was observed at 10°C for both solutions. Scattering intensity in the middle q range increased with lowering temperature due to the formation of ordered domains of associated helices. It should be noted that the maximum appeared in the profiles from gellan in 70 mM NaCl solutions by gelation, suggesting the

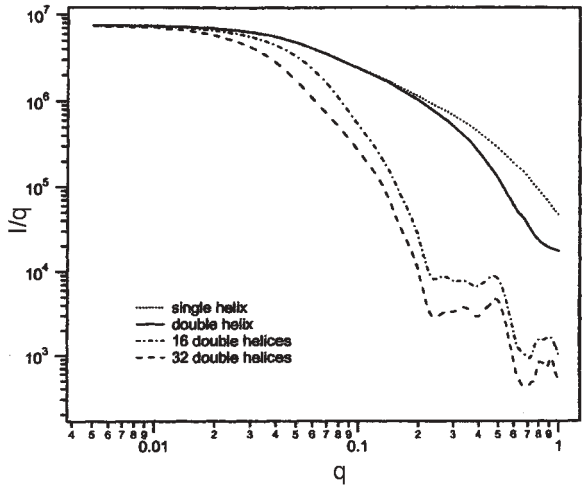

Fig. 9 The calculated scattering profiles corresponding to Fig. 7 and Fig. 8

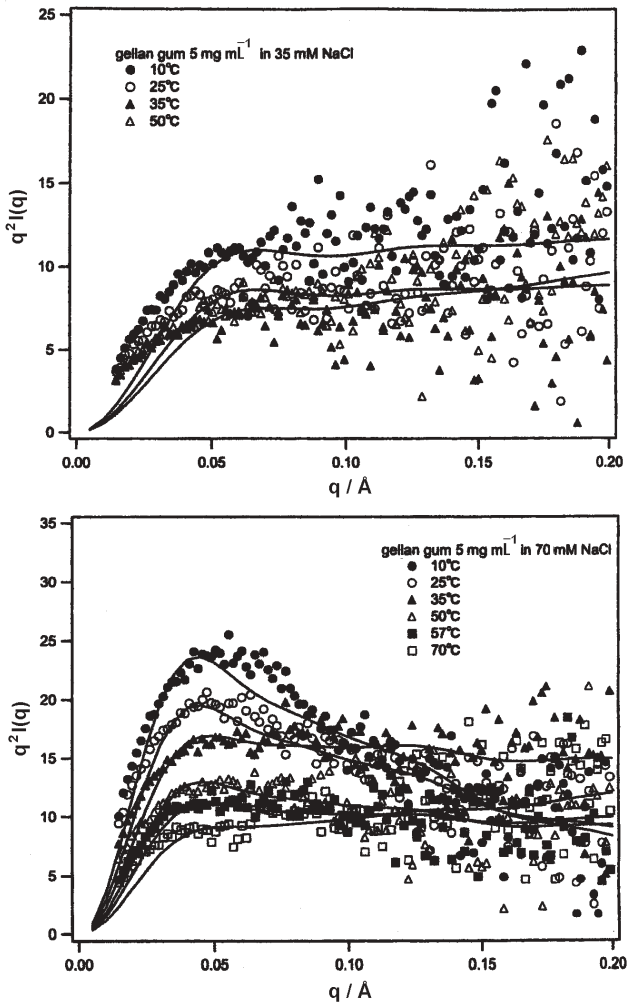

Fig. 10 The Kratky plots for the observed SAXS from gellan (5 mg mL^{-1}) in 35 and 70 mM NaCl aqueous solutions. The solid lines show the calculated SAXS

formation of well-defined domains. The solid lines represent the results fitted with the linear sum of scattering calculated from the molecular models as described above, and the fitted parameters were summarized in Table 1. The fitting was carried out by assuming two components of either 16 or 32 double helices representing a larger component and either single or double helix for computational simplicity, although a real system may contain various modes of associates distributed widely. In 35 mM NaCl solution the associated domain was found to be represented by 16 double helices at any temperatures, but the mass fraction of 16 double helices decreases by heating.

Table 1 Mass fraction of model components

Temperature/°C	In 35 mM NaCl	In 70 mM NaCl
10	16 double(76%)+double(24%)	32 double(97%)+double(3%)
25	16 double(77%)+double(23%)	32 double(96%)+double(4%)
35	16 double(72%)+single(28%)	32 double(90%)+double(10%)
50	16 double(72%)+single(28%)	32 double(93%)+single(7%)
57	–	32 double(91%)+single(9%)
70	–	32 double(85%)+single(15%)

In the similar manner the mass fraction of 32 double helices in 70 mM NaCl solution decreases by heating as shown in Fig. 11. The second component changed from a double helix to a single helix above the transition temperature evaluated by DSC measurements, confirming the observed DSC peak as being due to the helix-coil transition.

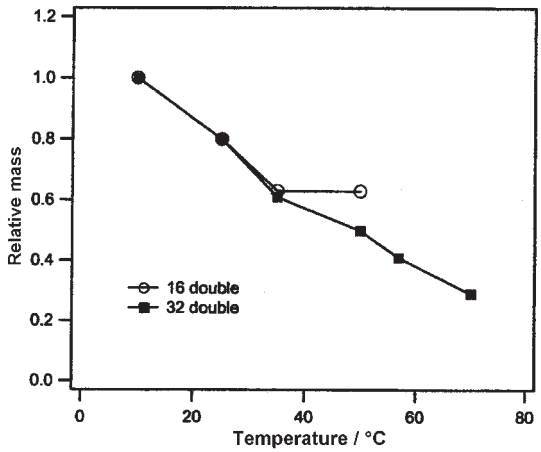

Fig. 11 The temperature variation of the mass fraction of aggregated domain models

Consequently the gelation of gellan in NaCl aqueous solutions takes place by the formation of double helices and the subsequent growth of the laterally associated domains, followed by the random aggregation of ordered domains. Since the SAXS exhibits no marked structural change corresponding to the higher peak in DSC profiles in the high concentration of salts, the transition observed as the second peak of DSC profiles is not due to the ordered-disordered structural change but due to the dissociation of random aggregates of ordered domains. That is, the gelation undergoes in two steps; first the conformational transition form single chain to double helix and subsequent alignment, and second the sol–gel transition at more macroscopic dimension where the ordered domains aggregate randomly to constitute the crosslinking domains for network formation.

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