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Description	

Ionic state and chain conformation for aqueous solutions of supergiant cyanobacterial polysaccharide

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We have investigated the electric conductivity, dielectric relaxation behavior, and viscosity for the aqueous solution of cyanobacterial megamolecules, molecular weight $= 1.6 \times 10^7$ g/mol, named sacran. Sacran is an anionic polyelectrolyte which has carboxylate and sulfate groups on the saccharide chain. The electric conductivity and the zero shear viscosity demonstrated three crossover concentrations at 0.004, 0.02, and 0.1 wt%. The viscosity was found to be scaled as $\sim c^{1.5}$, $\sim c^{0.5}$, $\sim c^{1.5}$, and $\sim c^{3.0}$ with increasing the sacran concentration. At 0.1 wt%, the sacran chain formed a weak gel which exhibits macroscopic liquid crystal domains including Schlieren texture. Therefore, these crossover concentrations are considered to be the overlap concentration, entanglement concentration, and gelation concentration (or critical polyelectrolyte concentration), respectively. Dielectric relaxation analysis exhibited the fact that sacran has two types of counterions with different counterion-polyion interaction, i.e., strongly bound and loosely bound counterions. The dielectric parameters such as relaxation time or relaxation strength are sensitive to both the entanglement concentration and the gelation concentration, but not the overlap concentration. The number density of bound counterions calculated from the relaxation strength revealed that the counterion is condensed on the sacran chain with raising the sacran concentrations. The decrease in the charge density of the sacran chain reduces the repulsive force between the chains and this would cause the helix transformation or gelation behavior. The chain conformation of sacran in pure water and the gelation mechanism are discussed in relation with the behavior of polyelectrolytes and liquid crystals.

I. INTRODUCTION

Sacran is a giant polysaccharide with a molecular weight of 1.6×10^7 g/mol which is extracted from the jelly extracellular matrix of a river plant named *Aphanothece sacrum*. We have investigated the molecular structure and swelling property [1], liquid crystalline (LC) behavior [2], and ion adsorption behavior [3–5] of the aqueous sacran solutions. The saccharide chain contains carboxylate groups (17 mol%) and sulfate groups (12 mol%) relative to the sugar residues, that is, sacran is a heteroanionic polyelectrolyte. Differently from celluloses, sacrans are heteropolysaccharides composed of various sugar residues such as Glc, Gal, Man, Xyl, Rha, Fuc, GalA (anionic), and GlcA (anionic), with a composition of 25.9%, 11.0%, 10.0%, 16.2%, 10.2%, 6.9%, 4.0%, and 4.2%, and contain trace amounts ($\sim$1.0%) of Ara, GalN (cationic), and Mur (amphoteric). Transmission electron and atomic force microphotographs exhibit that the length of sacran chains attains 8 μm. Sacran shows high absorption efficiency not only for water (6100 ml/g) but also for 0.9% saline (2700 ml/g). Sacran chains form double helixes at concentrations $c > 0.09$ wt% and form a weak gel at $c > 0.25$ wt%, and finally form huge domains of LC gels with centimeter scale at $c > 0.5$ wt%, which is quite a low concentration when compared to conventional LC polysaccharides. It was also revealed that sacran adsorbed trivalent ions of lanthanoid more efficiently than sodium alginate. It is quite natural that these characteristics strongly relate to chain properties such as

electric charges or the chain conformation. However, the chain conformation of sacran in pure water has not been clarified yet; therefore, the amazing phenomena mentioned above have not been understood clearly.

In the present study, we have tried to figure out the chain conformation and the state of electric charges of sacran in pure water by rheological and electrical measurements. It is well known that the concentration dependence of zero shear rate viscosity demonstrates a crossover when the chain conformation is changed. The dependency of the viscosity has been proposed by Dobrynin *et al.* [6] that is for the scaling for flexible polyelectrolytes. Here, the dependency of the viscosity for sacran is compared with the scaling theory; additionally, a comparison with the data of xanthan gum in pure water [7,8]—which is a big saccharide (2.0×10^6 g/mol) with electric charges—has been made. The state of electric charges for sacran chains can be detected by electrical measurements. The dc electric conductivity gives us information relating to the mobility of free counterions. The dielectric relaxation at the frequency range from kHz to MHz shows the information of bound counterions being affected by the electrostatic adhesive force due to polyions (sacran chain in this case). Generally in polyelectrolytes, strongly bound counterions and loosely bound counterions can be detected by the low-frequency relaxation (kHz range) and the high-frequency relaxation (MHz range), respectively. The strongly bound counterions fluctuate along the Coulomb-potential well on the polyion [9–11]; on the other hand, the loosely bound counterions fluctuate the average distance between polyions [10–12]. Considering the counterion condensation theory by Manning [13,14], it can be expected that the binding strength of the counterions directly affects the chain conformation. These electric properties—dc

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conductivity and dielectric relaxation—would be a powerful tool to understand the conformational transitions of sacran chains.

In the last section of this paper, we present a schematic illustration representing the conformation of the sacran chain in pure water by summarizing all of the rheological and electric aspects. We believe the illustration including the ionic state of the sacran chain will be helpful for understanding the solution properties of huge saccharide chains with electric charges.

II. EXPERIMENTAL PROCEDURE

A. Sacran extraction and solution preparation

Sugar chains were extracted from *Aphanothece sacrum* by the following procedure. The *Aphanothece sacrum* samples were freeze-thawed and washed in pure water, followed by lyophilization. The samples were washed three times using a large amount of ethanol with shaking (120 rpm) overnight, and then collected by filtration using gauze. The ethanol-washed samples were put into 0.1 M NaOH aq. at $100\,^{\circ}$C, and agitated at constant temperature for 4 h to yield the transparent solution. The solution was dialyzed with pure water for more than 72 h using the regenerated cellulose membrane (MWCO: 14000) until the pH value decreased to 8.0–9.0, and was then filtrated. Then the filtrate was concentrated by a rotary evaporator to create a highly viscous solution. The viscous solution was slowly poured into 100% isopropanol (1000 ml) to precipitate white fibrous material. The fibers were dissolved in hot water again, concentrated, and reprecipitated, and these operations were repeated three times in total. The fibrous precipitates in isopropanol were collected and dried using a vacuum oven. The dried sacran was completely dissolved in pure water at $100\,^{\circ}$C and was cooled at room temperature to obtain aqueous sacran solutions with 0.01, 0.1, and 1 wt%. The sacran solutions for electric and viscoelastic measurements were prepared by diluting the aqueous sacran solutions, and were heated again at $100\,^{\circ}$C for 30 min. The obtained aqueous solutions were reduced to room temperature and were provided for the measurements.

B. Electric and dielectric measurements

The electric conductivity and dielectric constant of sacran solutions were measured by an ac two-terminals method using an LCZ meter (HIOKI 3532-50) at $20.0 \pm 0.5\,^{\circ}$C. The frequency range was from 42 Hz to 5 MHz, and the applied voltage was 0.1 V. The sample cell used in the present study was a coaxial cylindrical condenser with stainless-steel electrodes. We poured 1 ml of an aqueous solution of sacran into the sample cell until the temperature reached the thermal equilibrium, followed by carrying out the conductivity measurement.

C. Rheological measurements

Viscoelastic measurements were carried out using a rheometer (MCR301, Anton Paar) with a stainless cone plate which has a diameter of 50 mm (CP50, Anton Paar). The minimum torque which can be detected by the rheometer is
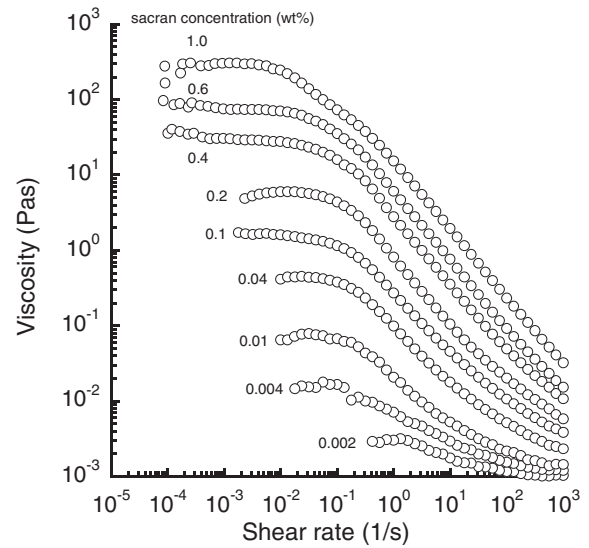

FIG. 1. Shear rate dependence of the apparent viscosity for sacran solutions at $25.0\,^{\circ}$C with various concentrations of sacran.

0.1 μN m. The temperature was controlled at $25.0 \pm 0.1\,^{\circ}$C using a Peltier plate during the viscoelastic measurement.

III. RESULTS AND DISCUSSION

A. Viscoelastic properties of sacran solutions

Figure 1 shows the shear rate dependence of the apparent viscosity for aqueous sacran solutions with various concentrations. The sacran solution demonstrated relatively high zero shear viscosity at 0.002 wt%, and high viscosity at concentrations above 0.1 wt%. A Newtonian plateau at low shear rates and shear thinning behavior at higher shear rates were observed for all sacran solutions. Interestingly, the shear thinning behavior was observed even for the extremely dilute solutions (e.g., 0.002 wt%). In general, the shear thinning behavior disappears in extremely dilute solutions. For example, a phosphate buffer solution of sodium hyaluronate does not exhibit shear thinning behavior below the overlap concentration although the hyaluronate has a long chain with a molecular weight of 1.6×10^6 g/mol [15]. However, there is a contradictory report [7,8] that a dilute solution of xanthan gum with a molecular weight of 2.0×10^6 g/mol exhibits the shear thinning behavior below the overlap concentration. Then, we analyzed the concentration dependency of the zero shear rate viscosity for sacran solutions.

Figure 2 shows the sacran concentration dependence of the zero shear rate viscosity η_0 for sacran solutions. Three critical concentrations appeared at 0.004, 0.015, and 0.20 wt%. In the dilute regime ($c < 0.004$ wt%), the viscosity of the sacran solutions was observed to be scaled as $\eta_0 \sim c^{1.5}$. The sacran solution demonstrated anomalous increase in the viscosity at 0.004 wt%. At 0.004–0.015 wt%, the viscosity showed a weak concentration dependence as $\eta_0 \sim c^{0.5}$. It is well known that the entire semidilute unentangled regime is well described by the empirical Fuoss law ($\sim c^{1/2}$). At 0.015–0.20 wt%, the viscosity increased as $\eta_0 \sim c^{1.4}$, which is very close to the scaling theory observed in the semidilute entangled regime ($\sim c^{3/2}$). Therefore, the sacran chain is considered to be in
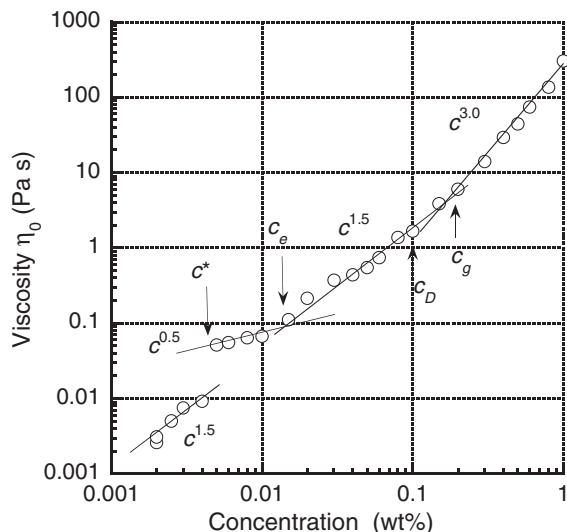
FIG. 2. Sacran concentration dependence of zero shear rate viscosity for sacran solution at 25.0 °C.

FIG. 3. Intrinsic viscosity for sacran solutions as a function of sacran concentration.

the dilute regime at $c < 0.004\%$, and behaves as an isolated chain. The region at $0.004 < c < 0.20$ wt% is considered to be the semidilute regime, which can be divided by the semidilute unentangled regime ($0.004 < c < 0.015$ wt%) and the semidilute entangled regime ($0.015 < c < 0.20$ wt%). It can be considered that the discontinuity point observed at 0.004 wt% is the overlap concentration c^*.

For polyelectrolytes without salt, the overlap concentration c^* can be explained as $c^* \sim a^{-3}N^{-2}$ using the Kuhn monomer size a and the number of Kuhn monomers in the chain N. In polyelectrolyte solutions without salt, the chain can be represented by the random walk of electrostatic blobs in which the constituent segment is stretched due to electrostatic repulsion and the segment behaves as a rigid rod. The overlap concentration for sacran was determined to be 1.4×10^{-8} wt% using the values of sacran $a = 0.65$ nm and $N = 8.9 \times 10^4$. This value is apparently lower than the experimental value; therefore, the sacran chain would not be fully extended in pure water in the dilute regime. For flexible polymers in a good solvent, the overlap concentration can be explained by $c^* \sim a^{-3}N^{-0.76}$. The overlap concentration was calculated to be 0.019 wt% using the above values of a and N, which is 4.8 times higher than the concentration of the discontinuity point observed. Therefore, the sacran chain in pure water is not fully extended like highly charged chains but collapsed compared to electrically neutral chains in a good solvent. The electrostatic repulsion between charged groups might not be sufficient for fully extending the sacran chain because it contains only carboxylate groups of 17 mol% and sulfate groups of 12 mol% relative to the sugar residues. Otherwise, the sugar unit with electric charges may be localized in the sacran chain; as a result the segment without electric charges would be shrunk as a neutral chain. Some of the anomalous behaviors seen in sacran solutions have been reported in the literature for xanthan solutions [7,8]; for example, a sudden increase in the shear viscosity at c^* concentration, a narrow region of the semidilute unentangled solution, and the shear thinning behavior below at c^* concentration. According to the literature, the overlap
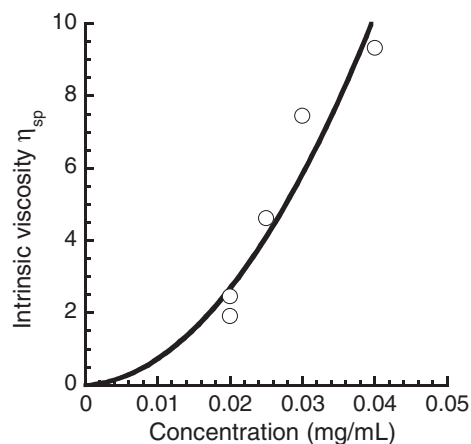
concentration c^* and the entanglement concentration c_e for xanthan solutions are 0.007 wt% and 0.04 wt%, respectively, which are similar to those of sacran solutions (c^*: 0.004 wt%, c_e: 0.015 wt%).

For dilute solutions ($c < c^*$), the specific viscosity η_{sp} can be described by the following Huggins equation [16]:

$$\eta_{sp}(c) \equiv \frac{\eta(c) - \eta_s}{\eta_s} = [\eta]c + k_H([\eta]c)^2 + \cdots, \qquad (1)$$

where η is the measured viscosity, η_s is the viscosity of the solvent, c is the polymer concentration, $[\eta]$ is the intrinsic viscosity, and k_H is the Huggins coefficient, respectively. Figure 3 represents the relationship between η_{sp} and c for sacran solutions in the dilute regime. As seen in the figure, the intrinsic viscosity is well fitted by the above equation, and the intrinsic viscosity and the Huggins coefficient were determined to be $[\eta] = 12$ ml/mg, and $k_H = 41$, respectively (η of pure water: 0.89 mPa s). Therefore, we get the values of $c^*[\eta] = 0.48$ and $\eta_{sp}(c^*) = 9.3$, which largely differs from an empirical relation of $\eta_{sp}(c^*) \sim 2$. An aqueous solution of xanthan also showed an anomalous value of $\eta_{sp}(c^*)$ of 90 [7]. The reason for high $\eta_{sp}(c^*)$ has not been clarified yet since there are only a few reports concerning the value of $\eta_{sp}(c^*)$ for polysaccharides in pure water.

To finish the rheological section, shall we discuss the chain conformation of sacran in the concentrated regime? It is widely accepted for an uncharged polymer in a good solvent that the viscosity can be scaled as $\eta_0 \sim c^{15/4}$ in the condensed regime. Above 0.20 wt%, the viscosity for sacran solutions was observed to increase as $\eta_0 \sim c^{3.0}$, indicating that the sacran can be regarded as an electrically neutral chain in a good solvent. Therefore, it can be considered that a crossover observed at 0.2 wt% is the critical polyelectrolyte concentration c_D. Actually, the viscosity of sacran solution in the presence of NaCl (100 mM) is equal to that of the sacran solution without salt. In addition, the conductivity for 0.1 wt% sacran solution in the presence of NaCl (100 mM) (11.2 ± 0.4 mS/cm) was equal to the sum of individual conductivity of the sacran solution and the salt solution (10.8 ± 0.5 mS/cm). When the concentration of sacran was higher than 0.2 wt%, the viscosity for the sacran solution increased by an addition of NaCl. Thus,
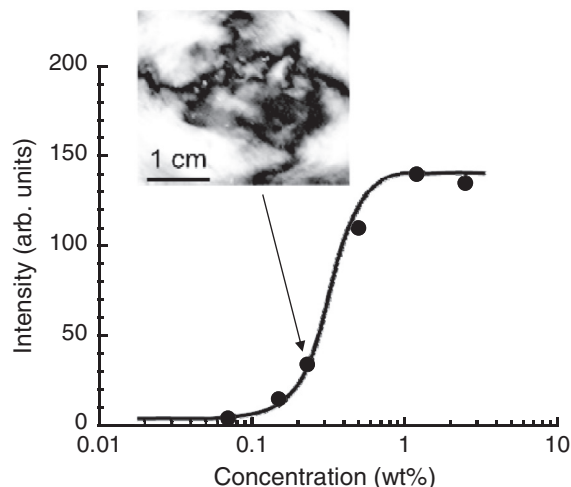
FIG. 4. Transmitted light intensity for sacran solutions as a function of sacran concentration. Inset shows a crossed-polarizing microscopic image.

the sacran is considered to be a condensed solution, and the chain behaves as an uncharged chain in a good solvent.

Figure 4 shows the transmitted light intensity of aqueous sacran solutions under the crossed polarizer as a function of sacran concentration. No transmitted light was observed below a concentration of 0.1 wt%; however, the light intensity gradually increased with the concentration. At 0.2 wt%, we recognized a small bright part in the scope and the light intensity increased remarkably at 0.5 wt%. This indicates that the sacran undergoes a structural transition from random to ordered structure due to the liquid crystalline interaction. The inset photo demonstrates the crossed-polarizing microscopic image of the sacran solution with 0.5 wt%. A bright macroscopic domain including Schlieren texture spreads over the whole sample, indicating that the sacran chain forms a nematic liquid crystal phase. The domain grows to a giant size with millimeter to several centimeter scales even without applying external shear [2]. According to the dynamic viscoelastic measurements, the storage modulus for a 0.25 wt% sacran solution was comparable to the loss modulus at wide frequencies of 0.01–10 Hz, suggesting the concentration is considered to be the gelation concentration. Summarizing all results in the condensed regime, the sacran chain loses its electric charges at 0.1 wt% and the chain would form a double helix by reducing the electric repulsive force arising on the saccharide chain (sacran chains form double helixes at concentrations $c > 0.09$ wt% as demonstrated previously [2]). Probably, the double helix makes a hydrophobic cross-linking point, and the cross-linking point grows to a giant and visible domain with liquid crystals.

B. Electric conductivity of sacran solutions

As described in the previous section, the chain conformation is strongly affected by the ionic state of the sacran chain, e.g., degree of dissociation of carboxyl or sulfonate groups. The information of the ionic state can be obtained mainly by the electric conductivity measurements. The electric conductivity of sacran solutions significantly varied with frequency at low
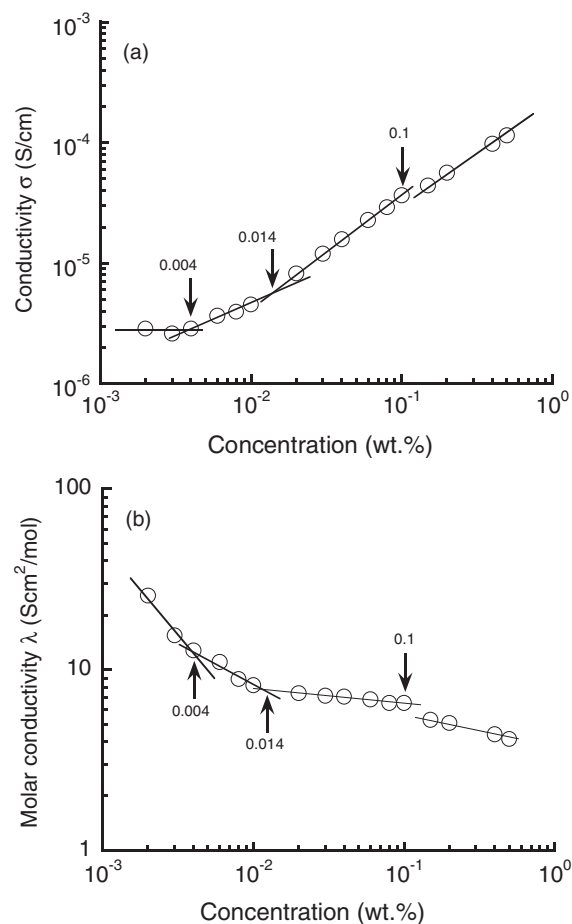

FIG. 5. (a) Electric conductivity at 100 kHz and (b) molar conductivity for sacran solutions as a function of sacran concentration.

and high frequencies; however, the frequency-independent conductivity was observed at frequencies around 100 kHz.

Figure 5(a) shows the electric conductivity at 100 kHz of aqueous sacran solutions as a function of sacran concentration. The electric conductivity for sacran solution increased with increasing the sacran concentration, and demonstrated two crossovers at 0.004 and 0.014 and a discontinuity point at 0.1 wt%. The discontinuity may be difficult to see because of the double logarithmic plot; however, the sacran solution certainly and reproducibly showed a remarkable decrease in the electric conductivity at 0.1 wt%. Figure 5(b) shows the molar conductivity for aqueous sacran solutions as a function of sacran concentration. The molar conductivity λ of the sacran solution can be calculated from the following equation:

$$\lambda = \frac{\sigma}{c}. \qquad (2)$$

Here, σ and c represent the electric conductivity and the molar concentration of the sacran solution, respectively. The molar conductivity significantly increased with decreasing the sacran concentration at concentrations below 0.014 wt%. According to the water retention experiment, sacran adsorbs a large amount of water (swelling ratio: ~6100 ml/g); however, the water retention significantly decreased at this concentration. It is empirically known that the degree of swelling of hydrogels largely increases with the degree of
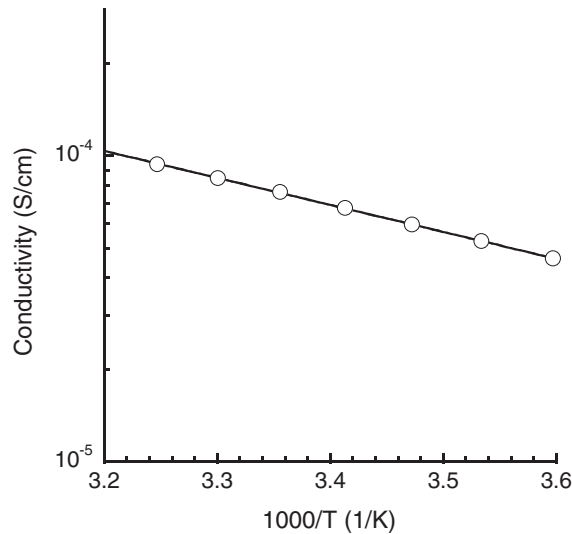
FIG. 6. Arrhenius plot of the electric conductivity at 100 kHz for 0.01 wt% sacran solution.

dissociation. The significant increase in the molar conductivity would be caused by the increase in the degree of dissociation of the carboxyl groups. As described in the rheological section, a conformational transition from semidilute unentangled to semidilute entangled regime occurs at 0.014 wt%. It is interesting that apparent changes in electric and water retention properties are observed at the transition from semidilute unentangled to semidilute entangled regime. At 0.1 wt%, the molar conductivity of sacran solutions discontinuity decreased from 8.0 to 5.8 S cm^2/mol, indicating that the free counterion of sacran contributing to the electric conductivity is further condensed on the sacran chain at this concentration. The sacran chain forms a helical structure at concentrations above approximately 0.1 wt% [2]. It can be considered that the sacran chain changes its conformation to a more compact structure by decreasing the electric charges on the sacran chain.

To find out the counterion species of sacran, we have measured the temperature dependence of the electric conductivity for aqueous sacran solutions. Figure 6 shows the Arrhenius plot of the electric conductivity for 0.1 wt% aqueous sacran solution. The electric conductivity can be well explained by the following Arrhenius-type equation:

$$\sigma = \sigma_0 \exp\left(-\frac{E}{k_B T}\right), \qquad (3)$$

where σ_0 is the conductivity factor; k_B, T, and E represent the Boltzmann constant, absolute temperature, and the activation energy, respectively. The activation energy of the electric conductivity was calculated to be 16.3 kJ/mol, indicating the counterion contributing the electric conductivity is Na$^+$ ion. If the counterion which contributes to the electric conductivity is a proton, the activation energy should be much smaller than the calculated value because of proton hopping via hydrogen bonds (Grothuss mechanism) [17]. We concluded that the counterion dissociated from carboxyl or sulfonate groups on sacran chain is Na$^+$ ion.
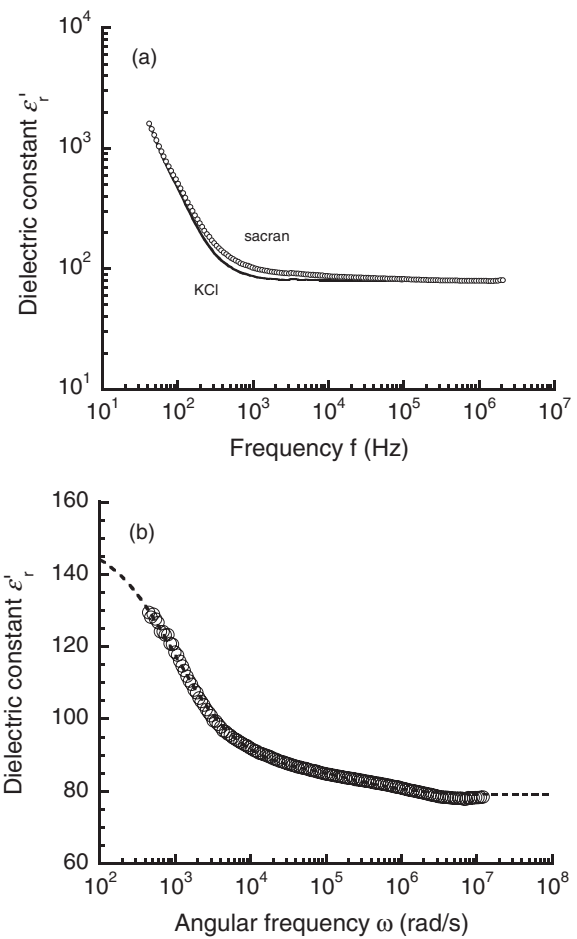

FIG. 7. (a) Frequency dependence of the real part of dielectric constant for 0.002 wt% sacran and KCl solutions. (b) The dielectric relaxation spectrum of 0.002 wt% sacran solution after subtracting the contribution of KCl solution. The broken line is a fit by Debye-type relaxations.

C. Dielectric relaxation behavior of sacran solutions

We have discussed, in the section on electric conductivity, the ionic properties of free counterions dissociated from macroions (sugar chain). In this section, the bound counterions in the vicinity of macroions are discussed. The ionic state of bound counterions can be detected by the dielectric relaxation measurements at low frequencies. Figure 7(a) shows the frequency dependence of the as-measured dielectric constants (real part) for 0.002 wt% sacran solution ε'_{am} and aqueous KCl solution ε'_{KCl}. The KCl solution was used as a reference solution to estimate the electrode polarization effect [18]. This subtraction method has limitations in the sense that the effects are not necessarily the same for the sample and reference solutions [19,20]. On the other hand, there are some papers describing the dielectric relaxation of DNA [21–23] and hyaluronic acid solutions [24] using this method. It cannot be denied that their dielectric data gave us important knowledge to understand the function of biopolymers. In this study, as a first step to investigate the ionic state of sacran, we employed the subtraction method using KCl solutions which have the same conductivity with sacran solutions. The net dielectric constant

of sacran ε'_s was calculated from the following equation:

$$\varepsilon'_s = \varepsilon'_{am} - \varepsilon'_{KCl}. \tag{4}$$

Here, ε'_{am} and ε'_{KCl} are the dielectric constant of as-measured data and KCl solutions, respectively. Two important facts which should be noted here were found on the dielectric spectra of the as-measured data. The first one is that the value of ε'_{am} was clearly higher than the value of ε'_{KCl} at 10^2–10^5 Hz, indicating the existence of the electric dipole due to bound counterions in sacran. The second is that the value of ε'_{am} at 10^6 Hz was 84, which is close to the value of $\varepsilon'_{KCl}(=84)$, indicating that no dielectric relaxation originating from the counterion polarization of the sacran molecule occurs at frequencies above 10^6 Hz. Figure 7(b) shows the dielectric relaxation profile of the sacran solution after subtracting the term of ε'_{KCl}. A large low-frequency relaxation and a high-frequency one were observed at frequencies around 100 Hz and 100 kHz, respectively. Although many semiempirical functions such as the Cole-Cole [25], Davidson-Cole [26], and Havriliak-Negami [27] functions have been used for the fitting of dielectric relaxation spectra, the observed dielectric relaxation can be successfully fitted to the sum of two Debye-type functions as follows:

$$\varepsilon'_s = \varepsilon'_w + \sum_i \frac{\Delta\varepsilon_i}{2} \left\{ 1 - \frac{\sinh x_i}{\cosh x_i + \cos(\pi/2)} \right\} \quad (i = 1,2), \tag{5}$$

where $x_i = \ln\omega\tau_i$, ω is the angular frequency of the applied electric field, and τ_i is the mean relaxation time. ε'_w is the dielectric constant of pure water (equal to 84), and $\Delta\varepsilon_i$ is the relaxation strength. The dotted line in the figure represents a result of fitting by Eq. (5). The correlation coefficients were higher than 0.999 for all fittings. The reason why the relaxations can be well fitted by the Debye-type function is unclear; however, it may originate from the spherical shape of the sacran chains.

Figure 8(a) shows the sacran concentration dependence of the relaxation time τ_1 for the low-frequency dielectric relaxation. Two crossover concentrations were observed at concentrations of 0.007 and 0.2 wt%. When the concentration was lower than 0.007 wt%, the relaxation time was in the order of $\sim$1 ms and was independent of the concentration. It has been widely accepted that the low-frequency relaxation of polyelectrolyte solutions is caused by the counterion fluctuation of strongly bound counterions along the Coulomb-potential well on the polymer chain [9–11]. The observed long relaxation time is one evidence that the sacran chain is extremely long. Such long relaxation time has been reported for a cross-linked polyelectrolyte gel (0.7–2.1 ms) [28] or Na-DNA ($\tau = 0.68$ ms: degree of polymerization 12 000) [21–24]. At $0.007 < c < 0.2$ wt%, the relaxation time decreased with concentration showing a power dependency as $\tau_1 \sim c^{-0.9}$ and subsequently was constant to be 40 μs at $c \geqslant 0.2$ wt%. Figure 8(b) shows the sacran concentration dependence of the relaxation time τ_2 for the high-frequency dielectric relaxation. The relaxation time τ_2 decreased with the concentration from 10 μs to 10 ns, and a slight change in the slope was recognized at 0.007 wt%. It has been widely accepted that the high-frequency relaxation of polyelectrolyte
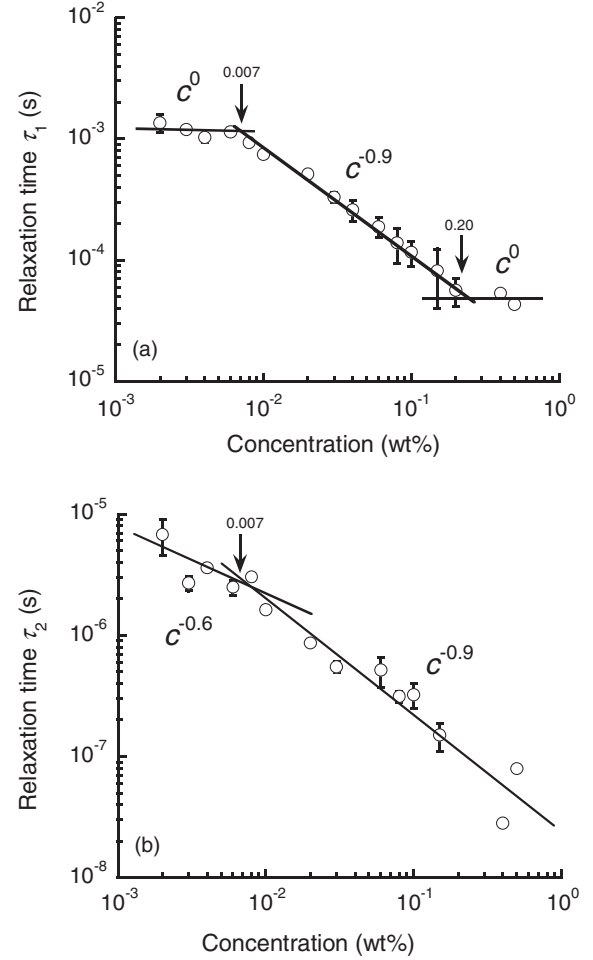

FIG. 8. Relaxation time of high- and low-frequency relaxations for sacran solutions as a function of sacran concentration.

solutions is caused by the counterion fluctuation of loosely bound counterions between polymer chains [10–12]. The concentration dependency of τ_2 was $\sim c^{-0.6}$ at $c < 0.007$ wt% and $\sim c^{-0.9}$ at $c > 0.007$ wt%.

The fluctuation length $\xi_{f,i}$ for the strongly bound ($i = 1$) and loosely bound ($i = 2$) counterions can be estimated from the mean relaxation time τ_i as follows:

$$\tau_i \cong \frac{\xi_{f,i}^2}{D} \quad (i = 1,2), \tag{6}$$

where D is the diffusion constant of counterions which is sufficiently well approximated by the diffusion constant of bulk ions [20,29]. Since the counterion of sacran is Na+, the diffusion constant of Na+ in a free medium ($=1.2 \times 10^{-9}$ m^2/s) was used for the calculation of both fluctuation lengths. The diffusion constant for strongly bound counterions may be much lower than that for loosely bound counterions. In this paper, the diffusion constants of strongly bound counterions and loosely bound counterions were not distinguished, similar to the analysis by Tomic *et al.* [21–23]. Using the mean relaxation time of τ_1 at $c < 0.007$ wt% ($=1.18 \times 10^{-3}$ s), the fluctuation length ξ_{f1} was determined to be 1.2 μm. Furthermore, the contour length of the sacran chain was estimated to be 3.8 μm using the theory by Mandel

($\xi_c \cong \sqrt{12}\xi_f$) [30], which coincides with the chain length obtained from AFM images [2]. At $c \geqslant 0.2$ wt%, the mean relaxation time was determined to be $\tau_1 = 5.07 \times 10^{-5}$ s, which gives a fluctuation length of 250 nm. This means that the strongly bound counterion fluctuates a limited part of the sacran chain probably due to the counterion condensation. The low-frequency relaxation time decreased as $\tau_1 \sim c^{-0.9}$, which gives the concentration dependence of the fluctuation length as $\xi_{f1} \sim c^{-0.45}$. This is consistent with the scaling theory, the de Gennes–Dobrynin renormalized Debye screening length, as $\sim c^{-1/2}$ [6,31,32]. The fluctuation length of the strongly bound counterions would be a function of the screening length. A similar phenomenon has been reported by Vuletić *et al.* that the fluctuation length for hyaluronic acid solutions in the semidilute regime was scaled as $\sim c^{-1/2}$ [24].

On the other hand, the high-frequency relaxation time decreased as $\tau_2 \sim c^{-0.6}$, which gives the relation between the fluctuation length and the sacran concentration as $\xi_{f2} \sim c^{-0.3}$. It is widely accepted that the high-frequency dielectric relaxation originates from the counterion fluctuation between the Coulomb-potential valleys on the charged chains (interchain fluctuation). Accordingly, in dilute solutions, the correlation length can be explained by the simple picture that a single chain of sacran occupies a spherical free volume with a diameter of the fluctuation length. Above the overlap concentration, the

high-frequency relaxation time decreased with the concentration as $\tau_2 \sim c^{-0.9}$, which gives the concentration dependence of the fluctuation length as $\xi_{f2} \sim c^{-0.45}$. Similar to the strongly bound counterions, the fluctuation length of the loosely bound counterions in a semidilute regime would be a function of the screening length.

Figure 9 shows the relaxation strengths $\Delta\varepsilon_1$ and $\Delta\varepsilon_2$ for the low- and high-frequency relaxations as a function of sacran concentration. A natural behavior was observed that the $\Delta\varepsilon_1$ increased in proportion to the sacran concentration at $c < 0.02$ wt%. The $\Delta\varepsilon_1$ took a constant value at concentrations of $0.02 < c < 0.2$ wt% although the concentration, i.e., the number of counterions, was raised. At $c > 0.2$ wt%, the $\Delta\varepsilon_1$ increased remarkably with the concentration. Similar behavior was observed for $\Delta\varepsilon_2$; however, the behavior at $c > 0.2$ wt% was opposite to that of $\Delta\varepsilon_1$. Combining with the relaxation time, the relaxation strength provides us quantitative information of bound counterions which contribute to the dielectric relaxations. Generally, the relaxation strength $\Delta\varepsilon_i$ due to counterion fluctuation can be written by the following formula:

$$\varepsilon_0 \Delta\varepsilon_i = \alpha_{e,i} \rho_{b,i} \sim \frac{e^2 \xi_{f,i}^2}{kT} \rho_{b,i} \quad (i = 1,2), \qquad (7)$$

where $\alpha_{e,i}$ and $\rho_{b,i}$ show the electrical polarization and the number density of bound counterions, e is the elementary charge, $\xi_{f,i}$ is the fluctuation length of bound counterions, k is the Boltzmann constant, and T is the absolute temperature.
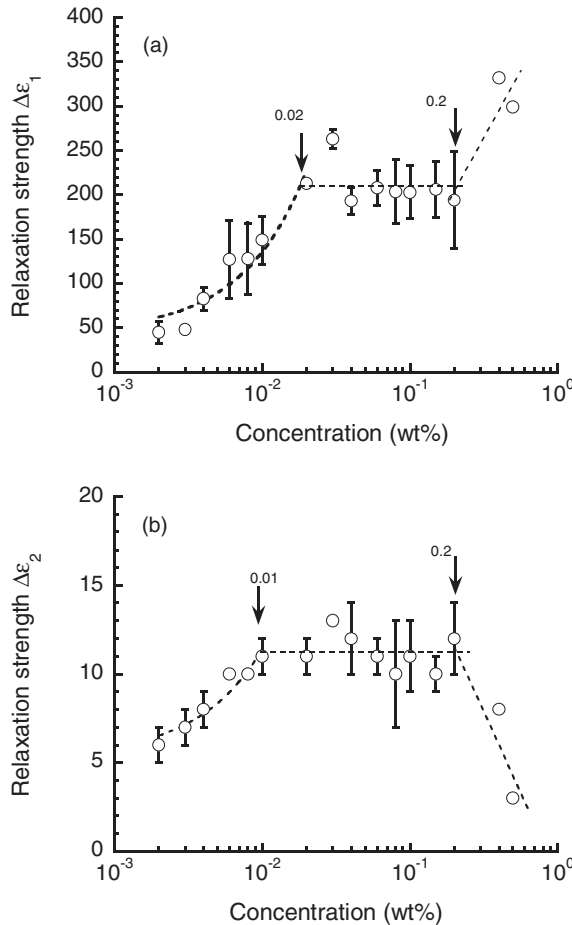

FIG. 9. Relaxation strength of high- and low-frequency relaxations for sacran solutions as a function of sacran concentration.
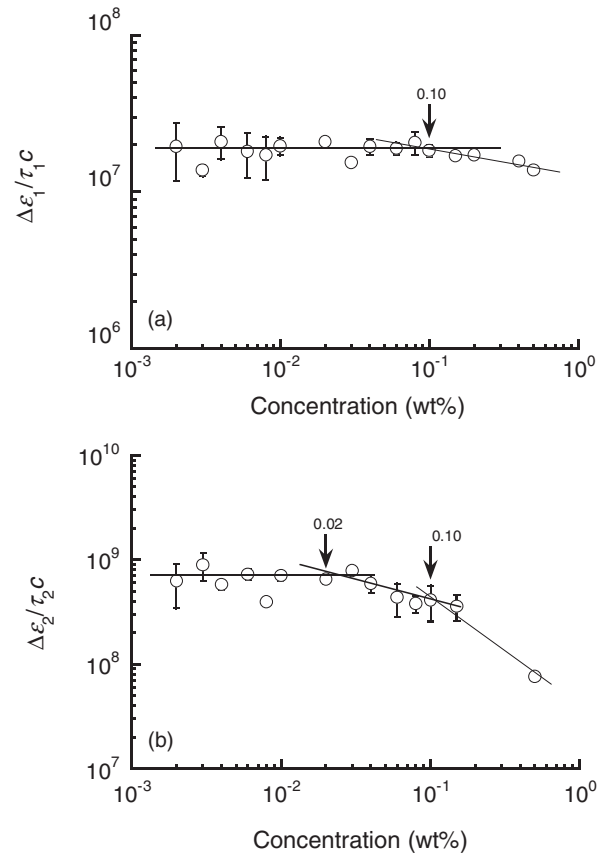

FIG. 10. $\Delta\varepsilon/\tau c$ for (a) strongly bound counterions and (b) loosely bound counterions for sacran solutions as a function of sacran concentration.
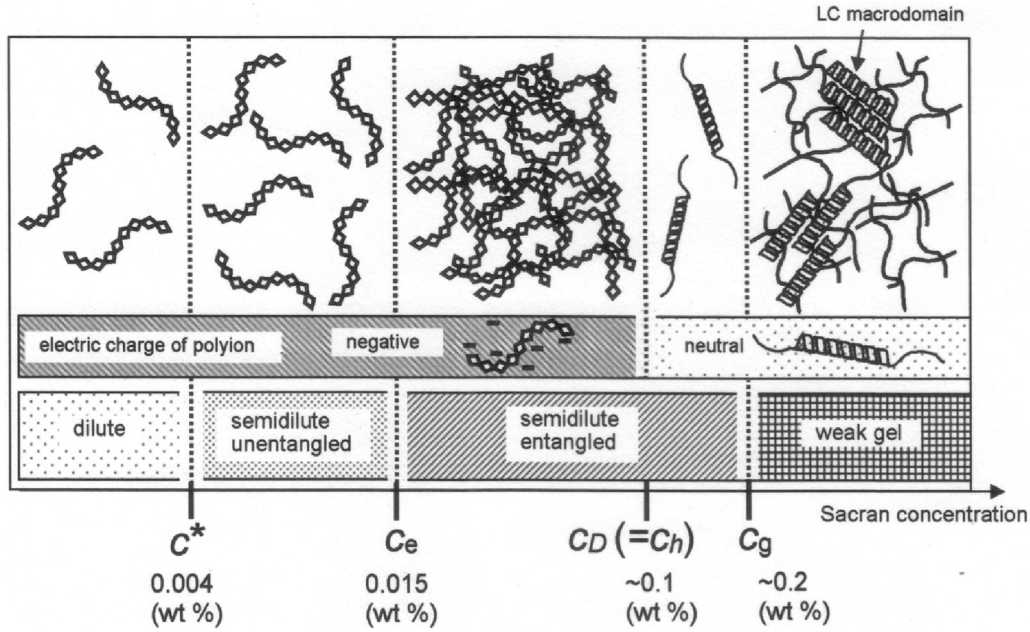
FIG. 11. Schematic illustrations representing the chain conformation of sacran in salt-free solutions [(c^*) overlap concentration, (c_e) entanglement concentration, (c_D) critical polyelectrolyte solution, (c_h) helix transition concentration, (c_g) gelation concentration].

From Eqs. (6) and (7), the number density of bound counterions can be estimated by the following equation:

$$\rho_{b,i} \sim \frac{\varepsilon_0 \Delta \varepsilon_i}{\tau_i} \quad (i = 1,2). \tag{8}$$

Figures 10(a) and 10(b) show the number density of bound counterions $\Delta \varepsilon_1 / \tau_1 c$ for observed two relaxations as a function of sacran concentration, assuming that the polarizability varies as the fluctuation length squared. The number density of strongly bound counterions $\Delta \varepsilon_1 / \tau_1 c$ was constant at $c < 0.10$ wt% and slightly decreased at high concentrations. On the other hand, the number density of loosely bound counterions $\Delta \varepsilon_2 / \tau_2 c$ was constant at $c < 0.02$ wt% and decreased showing a crossover at $c = 0.10$ wt%. Thus, the counterion condensation for both counterions is independent of the sacran concentration. This result roughly coincides with the data for DNA solutions [21,22] that the fraction of both counterions is independent of the DNA concentrations.

TABLE I. Characteristic concentrations determined by electric, dielectric, and viscosity measurements. "N.D." means "not detected."

Measurement	Parameter	c^{*a} (wt%)	$c_e{}^{b}$ (wt%)	$c_g{}^{c}$ (wt%)
Electric conductivity	σ	0.004	0.014	0.1
	τ_1	0.007	N.D.	0.2
	$\Delta \varepsilon_1$	N.D.	0.020	0.2
Dielectric relaxation	$\Delta \varepsilon_1 / \tau_1 c$	N.D.	N.D.	0.1
	τ_2	0.007	N.D.	N.D.
	$\Delta \varepsilon_2$	N.D.	0.010	0.2
	$\Delta \varepsilon_2 / \tau_2 c$	N.D.	0.020	0.1
Viscosity	η_0	0.004	0.015	0.2

[a]Overlap concentration.
[b]Entanglement concentration.
[c]Gelation concentration.

To summarize the electrical and rheological properties of aqueous sacran solutions, a schematic illustration representing the chain conformation of sacran in pure water is given in Fig. 11. The viscoelastic experiments and the scaling theory revealed three characteristic concentrations for the sacran solution; overlap concentration, entanglement concentration, and critical polyelectrolyte concentration (Table I). The sacran solution is in the dilute regime at $c < 0.004$ wt%. The extremely low value of the overlap concentration strongly suggests that the sacran is a giant molecule. The crossover concentration from the semidilute unentangled and the semidilute entangled regime is observed at 0.015 wt%. It should be noted that the electric conductivity and the shear viscosity are sensitive to the crossover concentrations. The transition from solution to weak gel occurs at approximately $c = 0.2$ wt%. This concentration corresponds to a characteristic concentration at which the sacran domains of the nematic LC phase are grown to a giant domain with sizes of several centimeters. It was also found from the salt effect on the viscoelasticity that the sacran chain loses its electric charges at $c \sim 0.1$ wt%; i.e., the characteristic concentration is the critical polyelectrolyte concentration. The molar conductivity supports this idea that the conductivity was found to decrease at $c = 0.1$ wt%. In the vicinity of the critical polyelectrolyte concentration, the sacran chain demonstrated to form double helix, that is, $c_D \sim c_h$ (c_h: the helical-transition concentration). As the sacran chain is neutral, two chains can be close to each other and form a double helix, which probably makes association points consisting of the giant domain; as a result a weak gel is formed.

IV. CONCLUSION

The electric and rheological properties of aqueous solutions of an anionic polysaccharide, sacran, have been investigated to clarify its chain conformation in pure water. The sacran

chain demonstrated extremely low overlap concentration of 0.004 wt% proving that the sacran is a giant molecule. The sacran chain in pure water is not fully extended like highly charged polyelectrolyte chains, but takes a collapsed conformation compared to electrically neutral chains in a good solvent. According to the viscosity measurement, the semidilute regime was found to be $0.004 < c < 0.2$ wt%, which can be divided by two regions of semidilute unentangled and semidilute entangled regime at 0.015 wt%. The whole semidilute regime—both the electric conductivity and dielectric relaxation behavior—revealed that the counterions dissociated from sulfonate and carboxyl groups are condensed on the sacran chain as the sacran concentration increases; as a result the charged density of the sacran chain decreases. At the concentrated regime, a helix transition was observed at 0.1 wt%, and a weak gel is formed at 0.2 wt%. Since the electric conductivity decreased discontinuously at the helix transition, the free counterions are much condensed on the sacran chain. A macroscopic domain of liquid crystals seen at $c > 0.2$ wt% is considered to be an aggregate of highly oriented helixes. Probably, the large domain of liquid crystals indicates that the persistence length (rigid part) of the helix is extremely long compared to the other polysaccharides. Thus, the conformational transitions are induced by the change in ionic state of the sacran chain which varies by the degree of condensation of counterions. Understanding the gelation mechanism of sugar chains with electric charges is important from a biological point of view. We believe that the findings obtained here are helpful for understanding the gelation mechanism of not only sacran but also charged polysaccharides.

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