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Research Article

Concentration Dependence of Zeta Potential and Electric Properties for Sacran and Xanthan Gum Aqueous Solutions

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Abstract

Sacran which is a giant polysaccharide with a molecular weight of 1.6×10^7 g/mol extracted from the jelly extracellular matrix of a river plant demonstrates interesting and anomalous properties originating from its extraordinary long chain with electric charges. In this study, we investigated the electric properties, zeta potential, electric conductivity, and dielectric constant, for sacran aqueous solutions to find out the ionic structure of counterions on the sacran chain, comparing with xanthan gum aqueous solutions. The zeta potential for 0.01 wt.% sacran and xanthan gum solutions was -90.1 and -77.0 mV, respectively, indicating that both polysaccharides have large negative charges on the saccharide chain. Upon increasing the sacran concentration, the zeta potential decreased and showed two characteristic concentrations at 0.11 and 0.20 wt.%, which corresponds to the helix transition concentration and gelation concentration, respectively. Similar behavior was observed for xanthan gum solution that two characteristic concentrations appeared at 0.10 and 0.30 wt.%, respectively. The dielectric constant at 1 MHz increased proportionally to the sacran concentration at regions below 0.11 wt.% and above 0.20 wt.%. At 0.11-0.20 wt.%, the dielectric constant was almost constant against the sacran concentration. The proportional coefficient for sacran solution was higher than that for xanthan gum solution at low concentrations, indicating large polarization of bound counterions of sacran. Additionally, the proportional coefficients below the helix transition concentration for sacran and xanthan gum were approximately 4-fold and 2-fold higher than those above the gelation concentration, respectively. The electric conductivity at 100 kHz for both polysaccharides increased proportionally to the sacran concentration below the helix transition concentration, however, it demonstrated under-deviation above the gelation concentration. It can be considered that the ratio of free counterions contributing to the electric conductivity decreased by the counterion condensation.

Keywords: Polysaccharide; Polyelectrolyte; Sacran; Zeta Potential; Dielectric Constant; Electric Conductivity

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 [1]. Sacran is an anionic polyelectrolyte which has carboxylate groups (17 mol%) and sulfate groups (12 mol%) relative to the sugar residues as shown in Figure 1 [2]. Transmission electron microscopy and atomic force microscopy revealed that the chain length of sacran attains 8 μm [1]. Owing to the extraordinary long chain, sacran shows anomalous properties, which are high water absorbency [3], efficient adsorption of rare earth metal ions [4-8], dispersing agent specially for multi-walled carbon nanotubes [9], anisotropic swelling of annealed sacran hydrogels [10], and instability-driven pattern formation in a limiting space [11-14]. Besides these features in physical properties, sacran demonstrates of interest and useful features in pharmacological, medical and biological sciences.

Arima has investigated the pharmacological efficacy and use of sacran in early stage and reported that sacran have a potential to improve atopic dermatitis through functional recovery of skin barrier and anti-inflammatory effects [15]. Motoyama., *et al.* found an exciting phenomenon that the anti-inflammatory effect is significant at a sacran concentration of 0.05 wt.% [16]. Ngatu., *et al.* found an inhibitory effect of sacran for 2,4,6-trinitrochlorobenzene (TNCB)–induced allergic dermatitis *in vivo* [17]. These efficacies

would be originated from the ionic structure (electrostatic interaction) or hydrophobic associations characteristic in sacran which is different from other polysaccharides since cells or tissues generally interact with charged groups or chains via hydration water. However, the molecular mechanism has not fully understood yet.

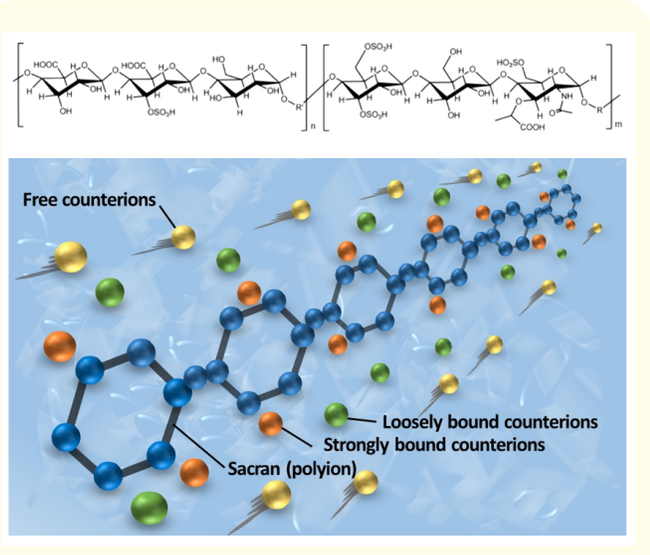

Figure 1: Schematic illustration of chemical structure for sacran (top) and ionic structure for sacran in pure water (bottom).

To elucidate the ionic structure of sacran, we have investigated so far the relationship between the chain conformation and ionic state of sacran in pure water by steady shear viscosity measurement, dynamic viscoelastic measurement, and electric measurement. It was revealed that sacran has two kinds of bound counterions, which are strongly bound and loosely bound counterions (Figure 1), due to the electrostatic interaction with anionic groups in the sacran chain [2]. Beside these bound counterions, there are free counterions contributing to the dc electric conductivity. In addition, we showed that sacran demonstrates four characteristic concentrations on the concentration dependence of shear viscosity, c* concentration (~0.004 wt%), overlap concentration (~0.015 wt%), helix transition concentration (~0.1 wt%), and gelation concentration (~0.2 wt%). Simultaneously, the electric properties such as molar conductivity or dielectric relaxation exhibited crossover points at these characteristic concentrations. This strongly indicates that the conformational change is intrinsically induced by the electrostatic interaction between the anionic groups on sacran chain and counterions dissociated from the anionic groups. However, the zeta potential reflecting to the effective electric charges on the sacran chain or the ability of electric polarization (electric polarizability) of bound counterions were not studied in the previous study. We consider the electrostatic interaction of bound counterions which are inner counterions localized at near the saccharide chain to be important for the elucidation of chain conformation of sacran in water.

In this study, we measured the zeta potential for sacran in pure water to clear the electric charge on the sacran chain and investigated the electrostatic interaction between counterions and the chain. Combining with the results of dc electric conductivity and dielectric constant, the effect of electrostatic interaction on the chain conformation is discussed. Similar measurements were carried out also for xanthan gum to compare with the electrostatic interaction for sacran.

Materials and Methods

Solution preparation

Polysaccharides were completely dissolved in pure water at 100°C and was cooled at room temperature to obtain aqueous solutions with concentrations of 0.1 and 1.0 wt%. Polysaccharide aqueous solutions for electric measurements were prepared by diluting these mother solutions. The diluted solutions were heated again at 100°C for 10 min before use and were reduced to room temperature and provided for the electric measurements.

Zeta potential measurement

The zeta potential was measured by a micro-electrophoresis zeta potential analyzer (Model 502 Japan Rufto Co., Ltd.) at room temperature (20 - 23°C). Sacran solution with 5 mL was poured into a cell with an electrode distance of 4.88 cm and an electric voltage of 49 V was applied to the electrodes. Image analysis was carried out using a software (Move-tr/2D Library Co. Ltd.) to analyze the movement of polysaccharides. The zeta potential at 20°C was determined from the observed value using an equation for temperature calibration.

Electric conductivity and dielectric constant measurements

The electric conductivity and dielectric constant for aqueous solutions of polysaccharides were measured by an ac two-terminals method using an LCR meter (IM3536 HIOKI) at 20.0 ± 0.5°C. The frequency range was from 60 Hz to 8 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 (SR-L1, Toyo Co.). An aqueous solution of polysaccharide with 1 mL was poured into the sample cell and it was placed in a thermostatic chamber with a temperature of 20.0 ± 0.1°C. The electric measurements were carried out after the temperature had reached a thermal equilibrium. The relative dielectric constant ε_r was calculated from

the capacitance using the dielectric constant in vacuum ε_0 as 8.85 $\times 10^{-14}$ F/cm.

Results and Discussion

Figure 2(a) shows the concentration dependence of zeta potential and microphotographs for sacran aqueous solutions. At c=0.01 wt%, the zeta potential was -90.1 mV and decreased with the sacran concentration. The zeta potential remarkably decreased at 0.11 wt% and was constant at 0.12-0.20 wt%, and then it decreased again at high concentrations. This means that the electric charge on the sacran chain reduces as increasing the sacran concentration. It has been reported [1] that the helix transition concentration for sacran in pure water is approximately 0.10 wt%. It can be considered that sacran forms helix by reducing the electric repulsive force between anionic groups on the chain. A further decrease in the zeta potential at c>0.20 wt% is probably caused by the counterion condensation.

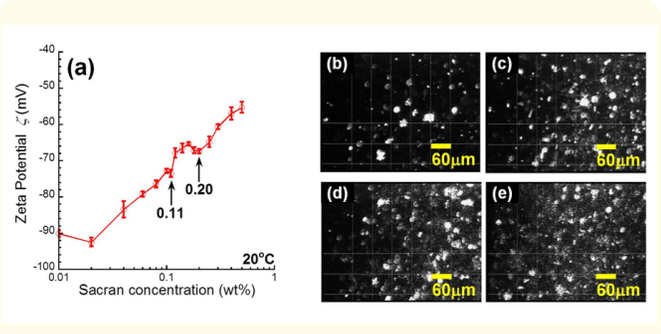

Figure 2: Sacran concentration dependence of (a) zeta potential and (b-e) microphotographs for sacran aqueous solutions. (b) 0.04 wt.%, (c) 0.14 wt.%, (d) 0.20 wt.%, (e) 0.50 wt.%.

Figure 3a indicates the concentration dependence of zeta potential and microphotographs for xanthan gum aqueous solutions. The zeta potential at c=0.01 wt% was -77.0 mV, indicating that the electric repulsive force between the chains for xanthan gum is weaker than that for sacran. The zeta potential decreased with the xanthan gum concentration and significantly decreased at 0.10 wt%. The zeta potential was constant at 0.16-0.30 wt% and decreased again at high concentrations. It has been reported [18] that the helix transition lies around 0.1 wt% although it depends on the molecular weight. Basically, the behavior of zeta potential for xanthan gum solution is similar to that for sacran solution. However, it is worth to mention that the decrease in the zeta potential at the helix transition was broader than that observed for sacran solution.

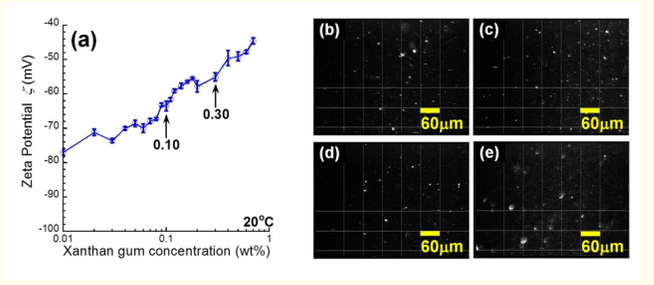

Figure 3: Xanthan gum concentration dependence of (a) zeta potential and (b-e) microphotographs for xanthan gum aqueous solutions. (b) 0.06 wt.%, (c) 0.10 wt.%, (d) 0.20 wt.%, (e) 0.40 wt.%.

Figure 4a exhibits the concentration dependence of relative dielectric constant at 1 MHz for sacran aqueous solutions. The dielectric constant for sacran solutions increased in proportional to the sacran concentration below 0.11 wt%, and it was almost constant at concentrations between 0.11 to 0.20 wt%. The dielectric constant increased again in proportional to the sacran concentration at high concentrations. The proportional coefficient α was calculated from the following equation

$$\alpha = \varepsilon_r / c \qquad (1)$$

Here, ε_r is the relative dielectric constant and c is the polysaccharide concentration. The proportional coefficient α was 55 at low concentrations (c<0.11 wt%) and 13 at high concentrations (c>0.20 wt%). This strongly indicates that the bound counterions of sacran can be largely polarized at low concentrations below the helix transition concentration.

Figure 4b demonstrates the concentration dependence of relative dielectric constant at 1 MHz for xanthan gum aqueous solutions. The dielectric constant for xanthan gum solutions increased in proportional to the xanthan gum concentration below 0.10 wt%, and it was constant at concentrations between 0.10 to 0.30 wt%. The dielectric constant increased again in proportional to the xanthan gum concentration at high concentrations. Similar to the sacran solutions, the proportional coefficient α for xanthan gum solutions was determined from eq. (1). The proportional coefficient α was 31 at low concentrations (c<0.10 wt%) and 15 at high concentrations (c>0.30 wt%). This strongly indicates that the bound counterions of xanthan gum can be largely polarized at low concentrations below the helix transition concentration. The value of α for sacran at above the gelation concentration was comparable with that for xanthan gum.

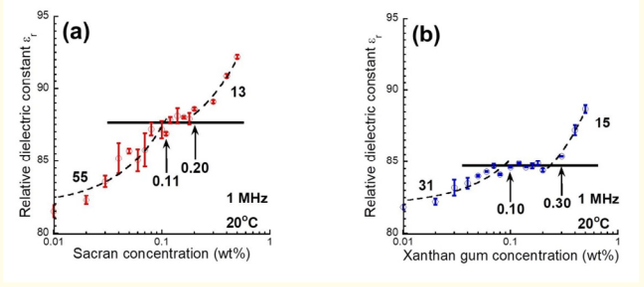

Figure 4: Polysaccharide concentration dependence of relative dielectric constant at 1 MHz for (a) sacran and (b) xanthan gum aqueous solutions.

Figure 5a and 5b show the concentration dependence of electric conductivity at 100 kHz for sacran and xanthan gum aqueous solutions, respectively. The electric conductivity increased proportionally to the polysaccharide concentration. The solid line indicates a fitting line where the conductivity is proportional to the concentration at c<0.11 wt.% for sacran and c<0.10 wt.% for xanthan gum. It was observed at above these helix transition concentrations that the electric conductivity was under-deviated from the fitting line. This strongly suggests that the ratio of free counterions contributing to the electric conductivity to the total ions decreased due to the helix formation. This understanding is in good agreement with the counterion condensation theory proposed by Manning [19,20].

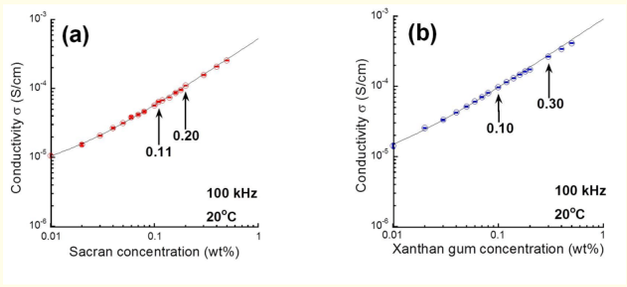

Figure 5: Polysaccharide concentration dependence of electric conductivity at 100 kHz for (a) sacran and (b) xanthan gum aqueous solutions.

The under-deviation of electric conductivity Δσ was determined from the following equation,

$$\Delta\sigma = \sigma_{calc} - \sigma_{obs} \qquad (2)$$

where the σ_{calc} and σ_{obs} are the calculated value and observed value of electric conductivity, respectively. The calculated value was obtained from the linear fitting shown in Figure 5. Figure 6 displays the polysaccharide concentration dependence of the deviation Δσfor sacran and xanthan gum aqueous solutions. Both of sacran and xanthan gum solutions, no decrease in the Δσ was observed at concentrations below approximately 0.1 wt%. A small decrease in the Δσ was observed at 0.2 wt% for sacran and 0.3 wt% for xanthan gum solutions, suggesting the counterion condensation. In addition, the decrease in the Δσ for xanthan gum was far higher than that for sacran. This strongly indicates that sacran does not change its ionic state while xanthan gum dramatically changes its ionic state to form helical structure.

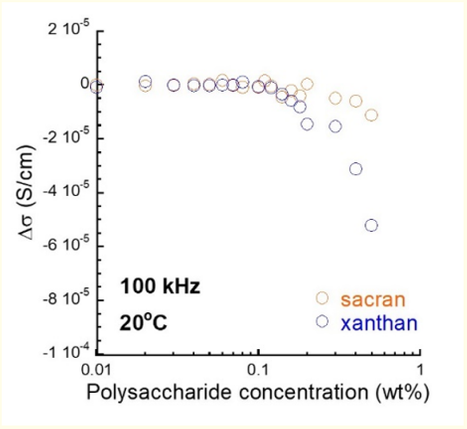

Figure 6: Polysaccharide concentration dependence of deviation between calculated and observed values of electric conductivity Δσ for sacran and xanthan gum aqueous solutions.

Conclusion

The ionic state for sacran and xanthan gum in pure water was investigated by the measurements of zeta potential, dielectric constant and electric conductivity. The zeta potential and the proportional coefficient of dielectric constant for sacran was apparently higher than that for xanthan gum, indicating that the sacran is a rod of polysaccharide with high electric charges. For both polysaccharides, the zeta potential decreased, and the dielectric constant increased with the polysaccharide concentration while showing a plateau region from the helix transition concentration to gelation concentration. This means that these electric measurements are useful to detect the helix transformation concentration of polysaccharides. The proportional coefficient for both polysaccharides showed that the electric polarizability significantly decreased at concentrations above the gelation concentration, suggesting that the strongly bound counterions are attracted on the polyion due to high ionic strength. It was also observed for both polysaccharides, the electric conductivity increased proportionally to the polysac-

charide concentration however it under-deviated at concentrations above the gelation concentration, indicating that the free counterions are condensed on the polyion at high concentrations. These electric properties strongly indicate that the sacran demonstrates a special ionic structure enabling high electric polarization below the gelation concentration. In contrast, there is no clear difference in the ionic structure between sacran and xanthan gum above the gelation concentration. We believe that these findings obtained here are useful for an efficient therapy for anti-inflammatory effects or allergic dermatitis through the polarized water molecules that strongly interact with the charged groups in sacran.

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Conflict of Interest

The authors declare no conflict of interest.

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