

Effects of monovalent cation and anion species on the conformation of gellan chains in aqueous systems

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Abstract

The effects of monovalent cation and anion species on the conformation of gellan chains in aqueous solutions were examined by optical rotation (OR) and fluorescence anisotropy measurements. The OR measurement suggested that a cation species with a large ionic radius aggregates the gellan chains to form supramolecular structures that have negative optical rotations even in the solution state. The anion species F^- lowered the coil–helix transition temperature and suppressed the aggregation of gellan chains, while the anion species Cl^- , Br^- , and I^- showed no influence on the coil–helix transition temperature or the aggregation behavior. The coil–helix transition of gellan chains was affected by the cation concentration rather than by the type of cation species; however, the latter was the predominant factor with respect to the aggregation of gellan chains.

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1. Introduction

Gellan is an anionic polysaccharide that contains a carboxyl group in each repeating unit (Fig. 1). Aqueous solutions of gellan form transparent, heat-resistant, and acid-resistant gels with a decrease in temperature (T) (Brownsey, Chilvers, Anson, & Morris, 1984; Jay et al., 1998). Gelation of gellan solutions is regarded to occur through a mechanism that involves a change in the conformation of gellan chains from random-coil to helix with a decrease in T . Further decrease in T leads to the aggregation of the helices to form the gel (Chandrasekaran, Puigjaner, Joyce, & Arnott, 1988; Crescenzi, Dentini, & Coviello, 1986; Grasdalen & Smidsrod, 1987; Matsukawa, Huang, & Watanabe, 1999; Matsukawa, Tang, & Watanabe, 1999; Robinson, Manning, & Morris, 1991). It has been reported that the physical properties of gellan gels are strongly affected by the type and concentration of added salt species (Chandrasekaran &

Thailambal, 1990; Crescenzi, Dentini, & Dea, 1987; Milas, Shi, & Rinaudo, 1990; Ogawa, 1999; Ogawa et al., 2001; Zasytkin, Braudo, & Tolstoguzov, 1997). The effects of the type of added salt species on the macroscopic properties of the gellan gels have been examined by thermal and mechanical measurements (Miyoshi, Takaya, & Nishinari, 1994, 1995, 1996; Moritaka, Nishinari, Taki, & Fukuba, 1995; Miyoshi, Nishinari, 1999; Nishinari, 1997). However, these effects have been insufficiently examined from the molecular point of view.

Optical rotation (OR) measurement is a powerful method for examining the helical conformation of polymer chains. Using this technique, we have examined the influence of salt concentration on the conformation of gellan chains in aqueous solutions and revealed that the salt concentration only affects the conformation of gellan chains in the helix state under the condition studied (Horinaka, Kani, Itokawa, Ogawa, & Shindo, 2004). Fluorescence anisotropy measurement is useful for evaluating polymer chain mobility, which is affected by the conformation of the polymer chain when it is isolated in solution, by the degree of interaction between the chains, and the conformation when it is aggregated. The mobility of gellan chains below the coil–helix transition temperature (T_{tr}) decreases with an increase in the salt concentration; however, the mobility of gellan chains in

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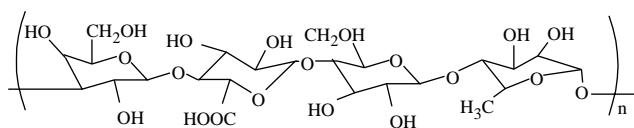


Fig. 1. Repeating unit of gellan molecules.

the random-coil state isolated in aqueous solution is not influenced by the salt concentration. These results indicate that the salt concentration does not influence the conformation of gellan chains in the random-coil state; however, the T_{tr} is clearly affected by the salt concentration.

In this study, the effects of the type of cation and anion species, derived from the added salt, on the conformation of gellan chains are examined by OR and fluorescence anisotropy measurements. The effects of the type of monovalent species on the T_{tr} and on the aggregation of gellan chains are separately discussed. Finally, we compare the effects of the type of cation species with those of the cation concentration.

2. Materials and methods

2.1. Sample preparation

Gellan (ELF7892, Wako, Japan) was purified three times by reprecipitation from water to 2-propanol. Table 1 shows the weight-average molecular weight (M_w) and the number-average molecular weight (M_n) of gellan obtained by GPC and the metal content of gellan estimated by the inductively coupled plasma method. Gellan labeled with fluorescein isothiocyanate (FITC) was used for the fluorescence anisotropy measurement. The preparation of labeled gellan is described in a previous paper (Horinaka, Kani, Honda, Uesaka, & Kawamura, 2004). Water purified by deionization after distillation was used as the solvent. Eight types of salts, namely, LiCl, NaCl, KCl, RbCl, CsCl, KF, KBr, and KI, were purchased from Wako and used without further purification. Aqueous gellan solutions were prepared as follows: gellan was immersed in water with salt for 6 h at 40 °C. Subsequently, the mixtures were stirred at 90 °C for 10 min to dissolve the gellan thoroughly. The gellan concentration of the aqueous samples used for the OR measurement was fixed at 0.2 wt%. The gellan samples used for the fluorescence anisotropy measurement contained 0.02 wt% labeled gellan and 0.18 wt% unlabeled gellan. We have represented the samples with the type of salt species and the salt concentration as shown in the following example:

Table 1
Characterization of the gellan sample

M_w	M_n	Metal content/wt%			
		Na	K	Ca	Mg
3.3×10^5	1.2×10^5	0.43	4.20	0.45	0.09

LiCl15 indicates that the gellan aqueous solution contains 15 mM LiCl as the added salt.

2.2. Optical rotation measurements

A polarimeter (J-500A, Jasco) was used for the OR measurement. It has been reported that gellan aqueous solutions that contain added salt form anisotropic gels at low temperatures. Further, the true OR value may be unobtainable by the conventional procedure due to the influence of anisotropy on the OR signal. We have developed an original measurement procedure, which cancels the influence of anisotropy of the sample on the OR signal. The OR values were acquired at every 20° during a 360° rotation by the alternating operations of the data acquisition and sample rotation, and the obtained 18 ($=360^\circ/20^\circ$) values were averaged. Since the signals induced by the macroscopic anisotropy of the sample vary sinusoidally with a period of 90° through the sample rotation, the signals can be eliminated by averaging the values obtained in the course of sample rotation to n times of 90° ($n=1,2,3,\dots$). Using this procedure, the true OR values were obtained even for anisotropic gellan gels (Horinaka, Kani, Honda et al., 2004; Horinaka, Kani, Itokawa et al., 2004). In this study, we employed this procedure for the samples in the gel state and the conventional procedure for the samples in the solution state (Horinaka, Kani, Honda et al., 2004; Horinaka, Kani, Itokawa et al., 2004). The OR measurement was conducted at 300 nm on cooling from 50 to 20 °C. Temperature was decreased at the rate of 0.5 °C/min and then maintained for 5 min prior to obtaining the OR values at each T . Eq. (1) was used to evaluate the specific rotation ($[\alpha]$) from the obtained OR value

$$[\alpha] = \frac{\phi}{cl} \quad (1)$$

where c , l , and ϕ are the concentration of the solution, the passlength of the cell, and the OR value, respectively.

2.3. Fluorescence anisotropy measurement

Fluorescence anisotropy measurement was conducted using a spectrophotometer (850, Hitachi). The excitation and emission wavelengths were set at 492 and 522 nm, respectively. The measurement was carried out with the same cooling process as that used for the OR measurement. Fig. 2 shows the definition of the measured values. The sphere located at the origin indicates the sample. The excitation light propagates in the $-Y$ direction and reaches the sample. The intensities of the vertical and horizontal components of the excitation light are denoted by I_V and I_H , respectively. The fluorescence propagating in the X direction is detected and the obtained fluorescence intensities are represented as I_{VV} , I_{VH} , I_{HV} , and I_{HH} ; I_{VV} and I_{VH} are the fluorescence intensities in the vertical and horizontal directions, respectively, when I_V is incident on

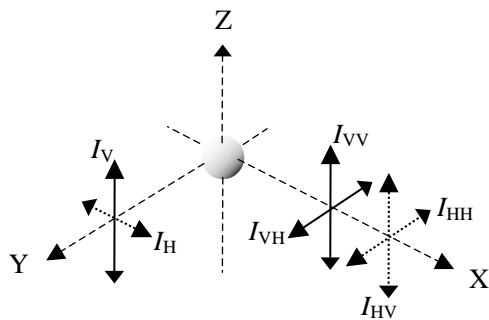


Fig. 2. The directions of I_V , I_H , I_{VV} , I_{VH} , I_{HV} , and I_{HH} with respect to the rectangular coordinates. The sphere located at the origin indicates the sample. The excitation light propagates in the $-Y$ direction, and the fluorescence propagating in the X direction is detected.

the sample. Similarly, I_{HV} and I_{HH} are the fluorescence intensities in the vertical and horizontal directions, respectively, when I_H is incident on the sample. The fluorescence anisotropy ratio (\bar{r}) is defined by Eq. (2). The constant G is defined by Eq. (3), and it is the apparatus constant that accounts for the sensitive ratio of the detection system

$$\bar{r} = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}} \quad (2)$$

$$G = \frac{I_{HV}}{I_{HH}} \quad (3)$$

Eq. (2) indicates that the \bar{r} value decreases when I_{VV} and I_{VH} are close to each other. This is the case when the mobility of gellan chains is high; thus, the chain mobility is estimated from the \bar{r} value.

3. Results and discussion

3.1. Effect of cation species

Fig. 3 shows the T -dependence of the OR values for LiCl15, NaCl15, KCl15, RbCl15, and CsCl15. None of these samples formed a gel within the T range examined. The T -dependence is qualitatively similar among the cases studied. The $[\alpha]$ value decreases with a decrease in T ; however, it starts increasing at approximately 30 °C. This inflection point probably corresponds to the T_{tr} of gellan chains from random-coil to helix, as previously reported (Horinaka, Kani, Honda et al., 2004; Horinaka, Kani, Itokawa et al., 2004; Rees, Scott, & Williamson, 1970; Stevens & Sathyanarayana, 1988). The T_{tr} values for these samples are 28 °C for LiCl15, 29 °C for NaCl15, 30 °C for KCl15, 30 °C for RbCl15, and 30 °C for CsCl15. In the case of gellan samples containing 30 mM salt, the T_{tr} values are 32.5 °C for LiCl30, 35 °C for KCl30 (data not shown), and >35 °C for CsCl30. These results indicate that the T_{tr} increases as the ionic radius of the cation species increases.

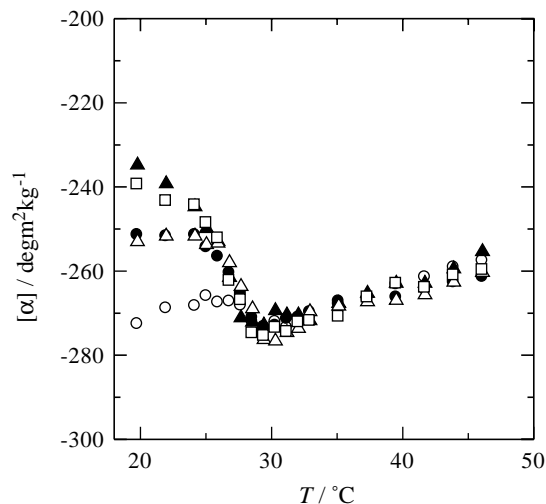


Fig. 3. The plots of $[\alpha]$ for gellan aqueous solutions: (▲) LiCl15; (□) NaCl15; (●) KCl15; (△) RbCl15; (○) CsCl15. The $[\alpha]$ values depend on the type of cation species at $T < T_{tr}$.

However, the difference in the T_{tr} among KCl15, RbCl15, and CsCl15 is unclear due to the low salt concentration. We were unable to determine the T_{tr} for CsCl30, because the $[\alpha]$ value decreases monotonously and the inflection point is unclear, as shown in Fig. 4. Judging from the observation that the gelation of CsCl30 occurred at 35 °C, the T_{tr} is expected to be higher than 35 °C.

The $[\alpha]$ profile below the T_{tr} can be categorized into three groups depending on the cation species added: Group A comprises LiCl15 and NaCl15 where the $[\alpha]$ values increase with a decrease in the T , Group B comprises KCl15 and RbCl15 where the $[\alpha]$ values increase with a decrease in the T and become almost constant below 25 °C, and Group C comprises CsCl15 where the $[\alpha]$ value increases with a decrease in the T and then decreases below 25 °C.

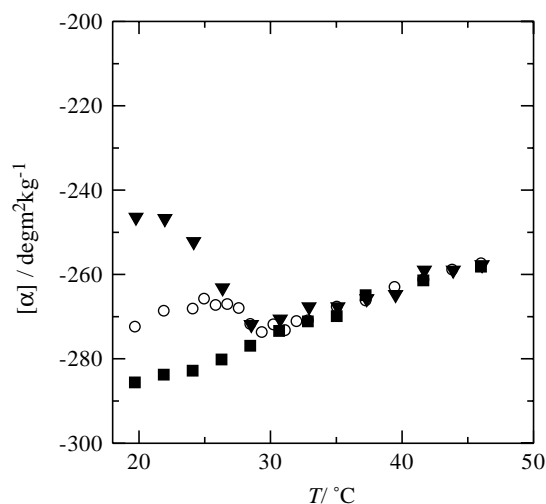


Fig. 4. The plots of $[\alpha]$ for gellan aqueous solutions: (▼) CsCl10; (○) CsCl15; (■) CsCl30. The $[\alpha]$ values decrease with increasing salt concentration at $T < T_{tr}$.

The increase in the $[\alpha]$ value just below the T_{tr} observed in all three groups is attributed to the increase in the helical content of gellan chains. This suggests that the helical content of the Group A samples increases monotonously with a decrease in the T . The decrease in the $[\alpha]$ value below 25 °C, which was observed for the Group C sample, can be explained by the decrease in the helical content of gellan chains or by the formation of a new conformation of gellan chains that has negative optical rotation, such as a supramolecular structure. We have reported that gellan chains that coexist with an added salt form supramolecular structures in the gel state, and these supramolecular structures have negative optical rotations (Horinaka, Kani, Honda et al., 2004; Horinaka, Kani, Itokawa et al., 2004). The decrease in the $[\alpha]$ value observed for the Group C sample may result from the microscopic aggregation that forms supramolecular structures even in the solution state. Viscoelastic measurements have also proposed that microaggregates exist in a 0.5 wt% aqueous gellan solution containing 10 mM salt (Miyoshi et al., 1994; Shoraku, Takigawa, & Masuda, 2002). The constant value of $[\alpha]$ observed for the Group B samples below 25 °C may be explained as the compensation of the increase in the $[\alpha]$ value due to the formation of helical structures, with the reduction in the $[\alpha]$ value caused by the formation of supramolecular structures.

The differences in the T_{tr} and the $[\alpha]$ profile below 25 °C can be explained by the difference in the ‘effective’ shielding ability of the different cation species for the electrostatic repulsion between the intramolecular segments and intermolecular chains. Gellan is an anionic polysaccharide, and when it is dissolved in aqueous solution, it has intramolecular and intermolecular electrostatic repulsions. When a salt is added in the solution, these electrostatic repulsions may be shielded by the cation species from the added salt. Comparison among the cation species implied that the difference in the ionic radius of the cation species results in the difference in their shielding ability. Cation species are hydrated with water molecules in aqueous solution and they form hydration shells. They also interact with counter ions such as anion species. Water molecules and anion species that surround the cation species suppress the shielding ability of the cation species; therefore, the strength of the ‘effective’ shielding ability can be determined by the strength of the interaction of the cation species with both the water molecules and the anion species. It was reported that the number of water molecules comprising the hydration shell (hydration number) differs among the cation species: it is 24.0 for Li^+ , 16.9 for Na^+ , 9.6 for K^+ , 6.4 for Rb^+ , and 3.7 for Cs^+ (Noyes & Falk, 1912; Smith, 1915). This suggests that a cation species with a smaller ionic radius attracts a larger number of water molecules, and the ‘effective’ shielding ability of such cation species for the electrostatic repulsions between the intramolecular segments and intermolecular chains is lower.

Fig. 4 shows the $[\alpha]$ value for solutions with various CsCl concentrations. The T_{tr} values determined from the figure are 28 °C for CsCl10, 30 °C for CsCl15, and > 35 °C for CsCl30. The T_{tr} increases with an increase in the salt concentration. CsCl10 can be categorized in Group A, because the $[\alpha]$ values increase with a decrease in the T . The monotonic decrease in the $[\alpha]$ value for CsCl30 probably results from the gelation that occurred at a temperature close to T_{tr} ; therefore, the extent of decrease in the $[\alpha]$ value due to the intermolecular aggregation is greater than the increase in the $[\alpha]$ value due to the formation of the helical conformation. Although no increase in the $[\alpha]$ value for CsCl30 is observed, we categorize CsCl30 as well as CsCl15 into Group C because of the decrease in the $[\alpha]$ value with a decrease in temperature. This result indicates that the effect of the increase in the salt concentration is qualitatively similar to the effect of changing the cation species with another species of a larger ionic radius. This implies that the cation species with a larger ionic radius has a higher ‘effective’ shielding ability.

Fig. 5 shows the \bar{r} values for LiCl15, NaCl15, KCl15, RbCl15, and CsCl15. The \bar{r} value for each sample gradually increases with a decrease in T and shows a steep increase at around the T_{tr} determined from Fig. 3. The increase in the \bar{r} value indicates a reduction in the chain mobility, as described above. This reduction can be attributed to the conformational transition from the random-coil to helix, because the chain mobility of helices with ordered structures is more suppressed than that of random-coil chains.

At $T > T_{tr}$, where the gellan chains are in the random-coil state, the \bar{r} values are almost the same regardless of the cation species. This result suggests that the conformation of gellan chains in the random-coil state is unaffected by the cation species. At $T < T_{tr}$, a difference is observed in \bar{r} values; therefore, $\text{LiCl15} < \text{NaCl15} < \text{KCl15} < \text{RbCl15} < \text{CsCl15}$. This indicates that the mobility of gellan chains

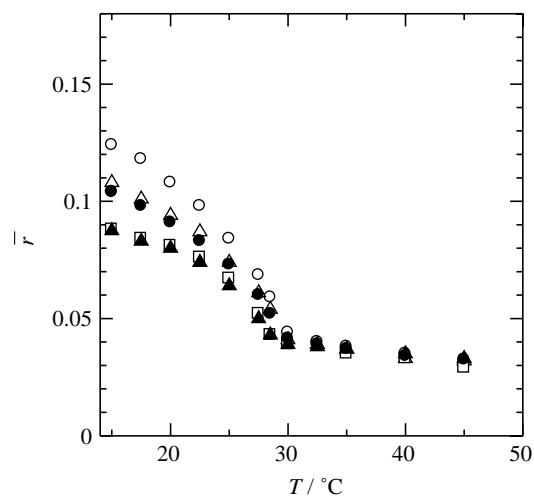


Fig. 5. The plots of \bar{r} for gellan aqueous solutions: (▲) LiCl15; (□) NaCl15; (●) KCl15; (△) RbCl15; (○) CsCl15. The \bar{r} values increase as the ionic radius of the cation species increases.

is in the order $\text{LiCl15} > \text{NaCl15} > \text{KCl15} > \text{RbCl15} > \text{CsCl15}$. The difference in the mobility below the T_{tr} can be explained by the difference in the degree of aggregation. As discussed for the result shown in Fig. 3, a cation species with a larger ionic radius aggregates the gellan chains more effectively. The mobility of a gellan chain in the aggregate is suppressed by steric hindrance of the surrounding gellan chains. Hence, the gellan chains in the sample that contains a cation species with a larger ionic radius have lower mobility.

3.2. Effect of anion species

Table 2 shows the $[\alpha]$ values at $T([\alpha]_T)$ and T_{tr} for KF15, KCl15, KBr15, and KI15. It appears that the values of $[\alpha]_{50}$, $[\alpha]_{20}$, and T_{tr} for KCl15, KBr15, and KI15 are determined regardless of the anion species. KF15 has a larger $[\alpha]_{20}$ value and lower T_{tr} than the other samples. Analogous to the effect of the cation species, the greater $[\alpha]_{20}$ value for KF15 may be explained as the weaker aggregation ability of KF for gellan chains than that of the other salt species. A comparison among the anion species indicates that F^- may suppress the aggregation of gellan chains. The anion species is also hydrated with water molecules in aqueous solution, and it interacts with the cation species. The strength of the negative electric effect of an anion species is determined by the strength of the interaction of the anion species with water molecules and cation species. The hydration numbers for the anion species F^- , Cl^- , Br^- , and I^- are 15.7, 9.6, 6.7, and 4.3, respectively (Noyes & Falk, 1912; Smith, 1915). These hydration numbers indicate that F^- attracts most of the water molecules surrounding it. The anion species F^- that has the strongest ability to attract water molecules may strongly interact with cation species in aqueous solution. Therefore, F^- decreases the cation concentration around the gellan chains and leads to the suppression of the shielding of the electrostatic repulsion between the gellan segments. The values of equivalent conductivity that reflect the mobility of ions also indicate the specific behavior of F^- ; the values of the equivalent conductivity for F^- , Cl^- , Br^- , and I^- are 46.7, 67.7, 66.6, and 65.5, respectively (Noyes & Falk, 1912; Smith, 1915). The anion species F^- has lower mobility than the other anion species. This indicates that F^- attracts cation species as well as water molecules to form a larger cluster than the other anion species. We consider that since the K^+ concentration around gellan chains in KF15 is reduced by

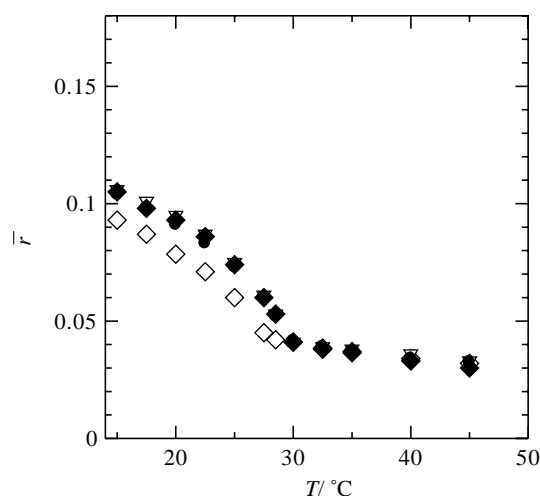


Fig. 6. The plots of \bar{r} for gellan aqueous solutions: (\diamond) KF15; (\bullet) KCl15; (\blacklozenge) KBr15; (∇) KI15. The \bar{r} value for KF15 is less than that of the other samples at $T < T_{\text{tr}}$.

F^- , the transition from the random-coil to helix and the aggregation of gellan chains in KF15 are suppressed in comparison with the other samples.

Fig. 6 shows the T -dependence of the \bar{r} value for KF15, KCl15, KBr15, and KI15. This figure shows that \bar{r} values in the random-coil state ($T > T_{\text{tr}}$) are very close to each other regardless of the anion species. The \bar{r} value for KF15 at $T < T_{\text{tr}}$ is smaller than that for KCl15, KBr15, and KI15. This small value for KF15 indicates that the mobility of gellan chains in KF15 is higher than that of the other samples at $T < T_{\text{tr}}$. As stated above, the anion species F^- attracts cation species and suppresses the aggregation of gellan chains. The high mobility of gellan chains in KF15 can be explained by the lower steric hindrance for the segmental motion of gellan chain due to the suppression of aggregation.

3.3. Comparison with the effects of salt concentration

In this section, we discuss the effects of the type of cation species on the T_{tr} and on the $[\alpha]_{20}$ value in comparison with the effects of the cation concentration. The T_{tr} value for LiCl30 obtained by the OR measurement is 33 °C (data not shown), which is higher than that obtained for CsCl15 (30 °C). This suggests that the conformational transition from random-coil to helix in LiCl30 with a decrease in T is easier than in CsCl15. As stated above, the T_{tr} for CsCl30 is higher than that for LiCl30 due to the higher shielding ability of Cs^+ ; however, in this case, the difference in the cation concentration predominates. The $[\alpha]_{20}$ values for LiCl30 and CsCl15 are -229 and -270 , respectively. This indicates that aggregation is easier in CsCl15 than in LiCl30. This is probably due to the higher shielding ability of Cs^+ in comparison with Li^+ . These results suggest that the coil-helix transition is greatly affected by the cation concentration, while the aggregation is mainly influenced by the type of cation species.

Table 2
 T_{tr} and $[\alpha]_T$ for gellan samples with various types of anion species

	T_{tr}	$[\alpha]_{20}$	$[\alpha]_{50}$
KF15	27	-241	-262
KCl15	30	-251	-263
KBr15	30	-253	-259
KI15	30	-254	-261

The T_{tr} values for KF15 and LiCl15 are 27 and 28 °C, respectively; therefore, LiCl15 has a higher T_{tr} than KF15 although Li^+ has a lower shielding ability than K^+ . As suggested above, the anion species F^- decreases the cation concentration around gellan chains. The lower cation concentration may result in a lower T_{tr} despite the type of the cation species. The $[\alpha]_{20}$ values for KF15 and LiCl15 are -241 and -234 , respectively, indicating that the type rather than the concentration of the cation species is predominant in the case of the aggregation, as stated above.

4. Conclusion

The effects of the cation and anion species on the conformation and mobility of gellan chains were examined by OR and fluorescence anisotropy measurements. Cation species that have a larger ionic radius have a higher 'effective' shielding ability for electrostatic repulsions between intramolecular segments and intermolecular chains. The fact that the $[\alpha]$ value decreased due to the formation of aggregates suggests that the aggregation of gellan chains results in the formation of supramolecular structures that have negative optical rotations. The anion species F^- suppresses the coil–helix transition and the aggregation of gellan chains, while there is no difference in the effect for the coil–helix transition and the aggregation between Cl^- , Br^- , and I^- . Based on the comparison between the T_{tr} and the $[\alpha]_{20}$ value, it is concluded that the coil–helix transition is greatly affected by the effect of cation concentration, and the aggregation is greatly influenced by the effect of cation species.

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