ELSEVIER

Biophysical Chemistry 111 (2004) 223–227

Biophysical Chemistry

www.elsevier.com/locate/bpc

Effect of pH on the conformation of gellan chains in aqueous systems

Jun-ichi Horinaka[a,*], Kohei Kani[a], Yuko Hori[b], Shiro Maeda[b]

[a] Department of Material Chemistry, Graduate School of Engineering, Kyoto University, A3-111, Takigawa laboratory Kyotodaigak, Nishikyo, Kyoto 615-8510, Japan
[b] Department of Applied Chemistry and Biotechnology, Faculty of Engineering, Fukui University, Bunkyo, Fukui 910-8507, Japan

Received 19 May 2004; accepted 8 June 2004

Abstract

The optical rotation and fluorescence anisotropy for gellan aqueous systems were measured at pH 4, 7, and 10 to elucidate the effect of pH on the conformation of gellan chains. The optical rotation study suggests that pH affects the conformation of helical gellan chains and their aggregation behavior but the coil–helix transition temperature. By comparing the chain mobility estimated from the fluorescence anisotropy between different pH conditions, it has been revealed that the degree of expansion of random-coiled gellan chains varies with pH. These results indicate that the effect of pH is explained by the change in the anionic nature of gellan chains rather than in the shielding effect of hydrogen ions surrounding gellan chains as a cation species.

Keywords: Gellan; pH; Anionic chain; Cation; Conformation; Shielding effect

1. Introduction

Gellan has been widely used as a gel-forming material in the field of food science, because aqueous systems of gellan form edible gels at ambient temperature [1–12]. It is noteworthy that aqueous systems of gellan maintain the gel state over a wide range of pH in comparison with those of other polysaccharides. To utilize this ability of gelation, gellan gels have been prepared at various pH [1], and the effect of pH on the physical, for example the mechanical, properties of gels have been extensively examined [2–6]. Because these studies have been directed toward the application of gellan to gelling food materials, the understanding of the effect of pH is still insufficient from the molecular point of view. Previous studies have proposed that the effect of pH can be explained by the shielding effect of hydrogen ion (H^+) charged positive on the electrostatic repulsion between negatively charged carboxyl groups in gellan chains. This interpretation, arising in analogy with the effect of cation species, such as sodium ion (Na^+) and potassium ion (K^+), is verified in this study on the molecular level. The point is whether the effect of pH can be simply attributed to the change in concentration of H^+ that acts as a monovalent cation species like Na^+ and K^+. We estimated the conformation of gellan chains with optical rotation and fluorescence anisotropy measurements and compared them between different pH conditions. Gellan chains are considered in any one of three states in the aqueous systems, namely, random coils in solution, helices in solution, and helices in gel [7,8]. The conformational transition of gellan chains from random coil to helix occurs on cooling precedent to the gelation, and then the network of gellan gel is formed by the intermolecular aggregation of helical gellan chains on further cooling. The effects of pH on the conformation of gellan chains in each state and on the boundary conditions are discussed.

2. Experimental

2.1. Sample preparation

Gellan (ELF7892, Wako; Fig. 1) was three times purified by reprecipitation from water to 2-propanol. The molecular weight and metal content of the gellan sample are shown in Table 1. We also used a dye-labeled gellan (labeled-gellan)

---

* Corresponding author. Tel.: +81-7-53832455; fax: +81-7-53832458.
E-mail address: horinaka@rheogate.polym.kyoto-u.ac.jp (J. Horinaka).

0301-4622/$ - see front matter
doi:10.1016/j.bpc.2004.06.003

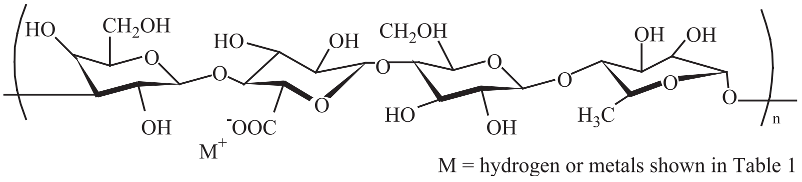

Fig. 1. Molecular structure of gellan.

for the measurement of fluorescence anisotropy. Fluorescein isothiocyanate (FITC; Wako) was labeled with the gellan chain via the reaction between cyanate group of FITC and hydroxyl groups in the gellan unit. The details of labeling reaction are described in the previous paper [13]. The labeled-gellan mixed with the unlabeled gellan at 10 wt.% was used for the measurement of fluorescence anisotropy. Water purified by distillation and deionization was used as the solvent, and the pH of gellan solutions was adjusted with the minimum of aqueous solution of HCl or NaOH to reduce the change in concentration of cation species in the solutions. The cation concentration was further stabilized with 15 mM of neutral salt, KCl, added into the solutions, whereby the change in cation concentration by the addition of HCl or NaOH ($\approx$ 0.1 mM) could be considered negligible in comparison with the concentration of cation species derived from 15 mM of KCl.

2.2. Measurement

Optical rotation was measured with an originally constructed apparatus consisting of a polarization modulation spectrometer (J-500A, JASCO) and a digital lock-in amplifier (SR830, SRS; [14]). The conventional and original measurement procedures were employed for nongelling and gelling samples, respectively [14]. The gelation was checked by observing the fluidity of the sample. The optical rotation was estimated in terms of the specific rotation ([α]), defined by,

$$[\alpha] = 100/\theta cl$$

where θ, c, and l are the measured rotation angle, concentration of solution, and path length, respectively. In this study, a cylindrical cell with 1 cm of path length was used.

Fluorescence polarization was estimated from the steady-state fluorescence intensities measured with a spectrophotometer (Type850, Hitachi) and polarizers. The excitation and emission bandpasses were settled at 492 and 522 nm, respectively. We confirmed that the fluorescence spectra for gellan solutions are independent of pH as well as concentration of gellan. Fluorescence anisotropy ratio ($\bar{r}$) is given as,

$$\bar{r} = \frac{I_{\mathrm{VV}} - GI_{\mathrm{VH}}}{I_{\mathrm{VV}} + 2GI_{\mathrm{VH}}} \tag{1}$$

$$G = \frac{I_{\mathrm{HV}}}{I_{\mathrm{HH}}} \tag{2}$$

where I_{XY} is the fluorescence intensity, and the subscripts represent the directions of linear polarization of the incident light (X) and emission (Y); for example, I_{VH} is the intensity of horizontally polarized component of fluorescence from the sample excited with vertically polarized light. The factor G accounts for the polarization bias of detection system. Fluorescence lifetime was measured with a time-resolved fluorescence spectrophotometer (OB920, Hamamatsu) using time-correlated single-photon counting method.

Table 1
Characterization of gellan sample

M_w[a]	M_na	Metal content/wt.%[b]			
		Na	K	Ca	Mg
3.3×10^5	1.2×10^5	0.43	4.20	0.45	0.09

[a] GPC analysis at 30 °C in aqueous solution with 1 g/l of NaCl.
[b] Measured with the ICP-AES method.

3. Results and discussion

Fig. 2 shows the plots of [α] against temperature (T) for 0.2 wt.% gellan solutions of pH 4, 7, and 10. The solutions did not form gels within the whole T-range examined. The

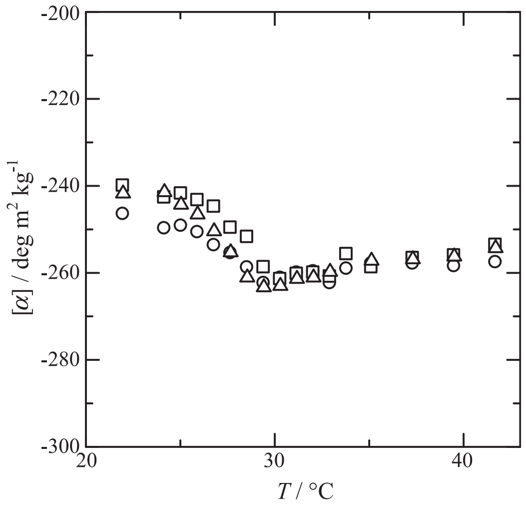

Fig. 2. Plots of [α] against T for 0.2 wt.% gellan samples with 15 mM of KCl at pH 4 (circle), 7 (triangle), and 10 (square). These samples does not form gel within the T-range examined in this study.

value of [α] slightly decreases with decreasing T and then turns to increase at an inflection point observed in the vicinity of 30 °C. The same profile of [α] was previously observed for other aqueous solutions of gellan, and it was concluded that the inflection point corresponds to the coil–helix transition temperature (T_{tr}) for gellan chains [14]. The close values of T_{tr} obtained from Fig. 2 suggest that the coil–helix transition of gellan chain is not significantly affected by pH of the solution. On the other hand, it was obtained that 0.2 wt.% gellan samples without KCl have the values of T_{tr} about 18 °C regardless of pH (data not shown), indicating that the coil–helix transition depends much on the concentration of KCl.

We see in Fig. 2 that the value of [α] at $T > T_{tr}$ does not depend on pH. Gellan chains are in random coil conformations in this T-range, and therefore, the comparable values of [α] results from the intrinsic optical activity of the monosaccharide unit constituting the gellan chain [14–16]. For $T < T_{tr}$, the pH dependence of [α] is observed; [α] decrease with decreasing pH, although the order observed in Fig. 2 is reversed at some temperatures. Because the change in [α] below T_{tr} implies the formation of helical conformations, the difference in [α] suggests that the helical conformation is affected by pH.

The effect of pH on the conformation of helical gellan chains is further examined with 0.5 wt.% gellan samples. Fig. 3 shows the plots of [α] against T at pH 4, 7, and 10. The systems of pH 4 and 7 formed gels in the low T region. The phase boundaries between solution and gel states are indicated by dotted lines in Fig. 3. The value of [α] shows clear pH dependence in the low T region, while [α] is independent of pH at $T > T_{tr}$. It is also noted that [α] for the gelling samples decreases with decreasing T in the gel state. It was reported that [α] for aqueous systems of gellan containing added salt decreases with decreasing T due to the negative optical rotation of supramolecular helical structure formed in the gel state [14]. The decrease in [α] observed in Fig. 3 for samples in the gel state can be similarly attributed to the intermolecular aggregation that forms a supramolecular structure having negative optical rotation. The value of [α] for gellan gels becomes smaller as pH decreases, which means that the ability to aggregate gellan molecules increases with decreasing pH. In this way, pH is likely to affect the intermolecular interaction of gellan chains. The pH dependence of [α] for 0.2 wt.% samples shown in Fig. 2 might be also attributed to the difference in the intermolecular aggregation, in addition to the difference in the helical conformation. Although the 0.2 wt.% samples do not form gel within the experimental condition employed, partial aggregation of gellan chains has been reported for nongelling gellan solutions [17,18]. It is impossible at present to estimate the two factors independently. We consider that both factors are responsible for the pH dependence of [α] at $T < T_{tr}$. The data supporting the contribution of both factors will be provided below.

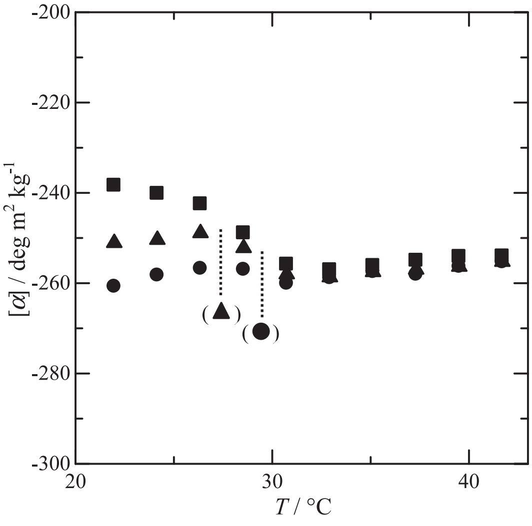

Fig. 3. Plots of [α] against T for 0.5 wt.% gellan samples with 15 mM of KCl at pH 4 (circle), 7 (triangle), and 10 (square). The samples at pH 4 and 7 form gel, and the phase boundaries between the solution and gel states are indicated by dotted lines with the symbols.

It was checked that the 0.5 wt.% gellan sample with pH 12 does not form gel within the T-range examined in this study. This result supports that the decrease in pH enhances the intermolecular aggregation of gellan chains. It was reported that the addition of cation species enhances the aggregation of gellan chains due to the shielding effect on the electrostatic repulsion of carboxyl groups included in gellan chains [8,11–13,17]. In addition, the ability to aggregate chains becomes greater as the size of cation species becomes larger. The order of [α] in the pH dependence shown in Figs. 2 and 3 could be explained by interpreting the change in pH as the change in the concentration of a cation species H^+. However, it is noteworthy that the sample of pH 12 includes 10 mM of Na^+ in addition to 15 mM of K^+ and 10^{-9} mM of H^+; namely, the total concentration of cation species in the sample of pH 12 is higher than that in the sample of pH 4 (15 mM of K^+ and 0.1 mM of H^+). Moreover, the sample of pH 12 contains larger ion species ($Na^+ > H^+$), and therefore, would have a higher ability for intermolecular aggregation. In fact, the aggregation of gellan chains is more enhanced at pH 4 than at pH 12; namely, the effect of pH on the intermolecular aggregation cannot be interpreted by the shielding effect previously reported for cation species. We attribute the ability of aggregating gellan chains at low pH to the charge density in the gellan chain. Because the carboxyl group included in gellan chain is a weak acid group, and the degree of dissociation of carboxyl groups in aqueous systems is dominated by the dissociation constant. The lower the value of pH becomes, the less fraction of carboxyl groups dissociates; namely, the gellan chains become less anionic polyelectrolyte as pH decreases. In this sense, the effect of pH on the gellan chain in aqueous systems is substantially different from the effect of ions such as Na^+ and K^+. The former changes the nature of the gellan chain, while the latter affects the surrounding condition of gellan chains. It is expected that the less anionic chains aggregate to one another easier because of the less electrostatic repulsion, and that this reduction effect by the change in the nature of chains may be greater than the shielding effect of surrounding cations. It should be noted again that the intramolecular conformational transition of gellan chain, namely T_{tr}, is likely to be dominated by the surrounding condition such as concentration of cation species, as stated above.

Fig. 4 shows the T-dependence of $\bar{r}$ for 0.2 wt.% samples both with and without KCl. The profile of $\bar{r}$ for samples with 15 mM of KCl is qualitatively similar to one another regardless of pH; the value of $\bar{r}$ slowly increases with decreasing T above 30 °C, and the increase in $\bar{r}$ becomes steeper at 30 °C. As shown in Fig. 2, T_{tr}s for these systems exist near 30 °C, suggesting that the change in the increasing rate of $\bar{r}$ at 30 °C is due to the coil–helix transition of gellan chains. We confirmed that the fluorescence lifetimes for the gellan systems employed in this study are almost constant against both pH and temperature within the experimental error, and that there is no interaction between fluorescent probes [13]. These results indicate that the increase in $\bar{r}$ is interpreted as the decrease in the chain mobility. The slow increase in $\bar{r}$ with decreasing T observed in Fig. 4 is attributed to the suppression of the thermal motion of gellan chains as well as solvent molecules, as is commonly observed for polymer chains in solutions [19–22]. The steep increase below 30 °C can be explained by the formation of helical conformations; the chain motion of helices with ordered structures may be more suppressed than that of random coil chains.

Comparing the values of $\bar{r}$ at $T>T_{\mathrm{tr}}$, we see that $\bar{r}$ at pH 4 is significantly larger than those at pH 7 and 10. The change in the solvent viscosity with pH is negligible within the condition employed in this study, indicating that the chain mobility at pH 4 is lower than that at pH 7 and 10. Because the random-coiled gellan chains are considered isolated in solution at $T>T_{\mathrm{tr}}$, this difference in the chain mobility is attributed to the intramolecular effect. As stated above, gellan chain becomes less anionic as pH decreases, and the decrease in the electrostatic repulsion between intramolecular segments may result in the suppression of chain expansion of gellan. It was reported that the chain mobility of nonelectrolyte polymers in solutions decreases as the chain expansion is suppressed [21,22]. We consider that this is also the case for gellan in aqueous systems. Previously, it was found that the expansion of random-coiled gellan chain is independent of the concentration of Na^+ [14]. By comparing $\bar{r}$ for samples of pH 4 between with and without KCl, it is also seen that the addition of K^+ does not affect $\bar{r}$ at $T>T_{\mathrm{tr}}$. These results mean that the change in the chain expansion with pH is due to the change in the anionic nature of gellan chain rather than the shielding effect of H^+ surrounding the gellan chain as a cation species. The values of $\bar{r}$ for pH 7 and 10 at a certain T are almost the same with each other. We consider that this is because of the small difference in the degree of dissociation of carboxyl groups. The pH dependence of $\bar{r}$ at $T<T_{\mathrm{tr}}$ is probably due to the difference in the size of aggregates of gellan chains and/or to the difference in the conformation of helical gellan chains, as stated above.

We compare the plots of $\bar{r}$ for 0.2 wt.% gellan samples without KCl at pH 4 and 12 shown in Fig. 4 to support the possibility that the conformation of helical gellan chains depends on pH as that of random-coiled chains. The values of T_{tr} for these samples were determined with optical rotation measurements to be ca. 17.5 and 25 °C, respectively, and are indicated with arrows in the figure. The sample of pH 12 has higher T_{tr} than that of pH 4 because of the higher concentration of cation species. The profile of $\bar{r}$ against T shows an inflection point, and the plot of $\bar{r}$ suddenly increases with decreasing T in the vicinity of T_{tr} for each sample. This behavior reflects the difference in the chain mobility between random coil and helix, as mentioned above. To discuss the conformation of helical gellan chains, the values of $\bar{r}$ for both samples are compared at 15 °C, where gellan chains exist as helices. Fig. 4 indicates that $\bar{r}$ for pH 12 is smaller than that for pH 4; namely, the helical gellan chains at pH 12 has higher mobility than those at pH 4. This suggests that the conformation of helical gellan chains is different between pH 4 and 12, although the data at low temperatures are insufficient.

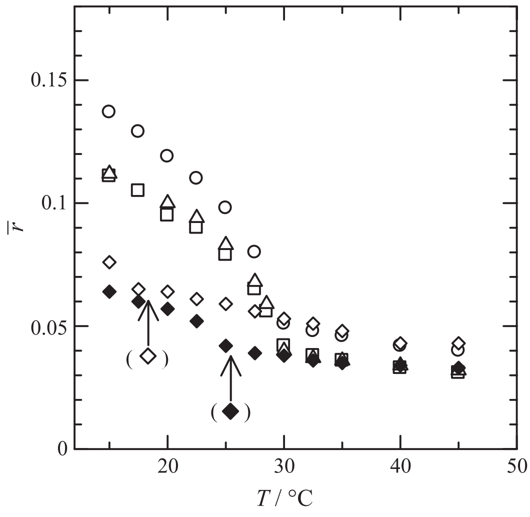

Fig. 4. Plots of $\bar{r}$ against T for 0.2 wt.% gellan samples with and without KCl. The symbols for samples with KCl are the same as those in Fig. 2. Diamond symbols represent the data for samples without KCl at pH 4 (unfilled) and 12 (filled). The arrows with the symbols in the figure indicate T_{tr} for samples without KCl determined by optical rotation study.

4. Conclusion

Optical rotation and fluorescence anisotropy were measured to estimate the effect of pH on the conformation of gellan chains in aqueous systems. The former elucidates the difference in the conformation of gellan chains in the helical sense, and the latter reflects the difference in the chain mobility. Gellan being an anionic polyelectrolyte, pH may affect the conformation of gellan chains in aqueous systems in two ways. One is the shielding effect on the electrostatic repulsion between carboxyl groups included in gellan units, as was reported for other cation species that affect T_{tr} and the intermolecular aggregation of gellan chains. The other one is the change in the anionic nature of the gellan chain determined by the degree of dissociation of carboxyl groups that varies with pH. It was obtained that the chain expansion of random coils is affected by pH, which is different from the fact that cations such as Na^+ and K^+ does not change the expansion of random-coiled gellan chains. Furthermore, it was suggested that the conformation of helical gellan chains also depends on pH. The intermolecular aggregation of gellan chains are enhanced by the decrease in pH more than by the increase in cation concentration. These results indicate that the conformation of gellan chains and intermolecular aggregation are dominated by the change in the anionic nature of gellan chains with pH.

References

[1] T.P. West, N.A. Fullenkamp, Effect of culture medium pH on bacterial gellan production, Microbios 105 (2001) 133–140.

[2] L.G. Fonkwe, G. Narsimhan, A.S. Cha, Characterization of gelation time and texture of gelatin and gelatin–polysaccharide mixed gels, Food Hydrocoll. 17 (2003) 871–883.

[3] H. Moritaka, K. Nishinari, T. Mika, H. Fukuba, Effect of pH, potassium chloride, and sodium chloride on the thermal and rheological properties of gellan gum gels, J. Agric. Food Chem. 43 (1995) 1685–1689.

[4] R. Mao, J. Tang, B.G. Swanson, Gelling temperature of gellan solutions as affected by citrate buffers, J. Food Sci. 64 (1999) 648–652.

[5] Y. Mo, K. Kubota, K. Nishinari, Rheological evidence of the gelation behavior of hyaluronan–gellan mixtures, Biorheology 37 (2000) 401–408.

[6] P.B. Deasy, K.J. Quigley, Rheological evaluation of deacetylated gellan gum (Gelrite) for pharmaceutical use, Int. J. Pharm. 73 (1991) 117–123.

[7] T. Hatakeyama, K. Nakamura, M. Takahashi, H. Hatakeyama, Phase transitions of gellan–water systems, Prog. Colloid & Polym. Sci. 114 (1999) 98–101.

[8] E. Miyoshi, K. Nishinari, Rheological and thermal properties near the transition of gellan gum aqueous solutions, Prog. Colloid & Polym. Sci. 114 (1999) 68–82.

[9] K. Nakamura, K. Harada, Y. Tanaka, Viscoelastic properties of aqueous gellan solutions: the effects of concentration on gelation, Food Hydrocoll. 7 (1993) 435–447.

[10] V. Carroll, G.R. Chilvers, D. Franklin, M.J. Miles, V.J. Morris, S.G. Ring, Rheology and microstructure of solutions of the microbial polysaccharide from *Pseudomonas elodea*, Carbohydr. Res. 114 (1983) 181–191.

[11] E. Miyoshi, T. Takaya, K. Nishinari, Gel–sol transition in gellan gum solutions: I. Rheological studies on the effects of salts, Food Hydrocoll. 6 (1994) 505–527.

[12] E. Miyoshi, T. Takaya, K. Nishinari, Gel–sol transition in gellan gum solutions: II. DSC studies on the effects of salts, Food Hydrocoll. 6 (1994) 529–542.

[13] J. Horinaka, K. Kani, H. Honda, Y. Uesaka, T. Kawamura, Local chain mobility of gellan in aqueous systems studied by fluorescence depolarization method, Macromol. Biosci. (submitted for publication).

[14] J. Horinaka, K. Kani, Y. Itokawa, E. Ogawa, Y. Shindo, Gelation of gellan gum aqueous solutions studied by polarization modulation spectroscopy, Biopolymers (in press).

[15] V. Crescenzi, M. Dentini, The influence of side-chains on the dilute-solution properties of three structurally related, bacterial anionic polysaccharides, Carbohydr. Res. 160 (1987) 283–302.

[16] P. Jansson, B. Lindberg, P.A. Stanford, Structure studies of gellan gum, an extracellular polysaccharide elaborated by *Pseudomonas elodea*, Carbohydr. Res. 124 (1983) 135–139.

[17] E. Ogawa, H. Matsuzawa, M. Iwahashi, Conformational transition of gellan gum of sodium, lithium, and potassium types in aqueous solutions, Food Hydrocoll. 16 (2002) 1–9.

[18] A. Shoraku, T. Takigawa, T. Masuda, Effects of alkaline metal salts on viscosity of gellan aqueous solutions, J. Soc. Rheol. Jap. 30 (2002) 13–17.

[19] J. Horinaka, M. Maruta, S. Ito, M. Yamamoto, Local motion of oligo- and polystyrene chain end studied by the fluorescence depolarization method, Macromolecules 32 (1999) 1134–1139.

[20] D.B. Adolf, M.D. Ediger, T. Kitano, K. Ito, Viscosity dependence of the local segmental dynamics of anthracene-labeled polyisoprene in dilute solution, Macromolecules 25 (1992) 867–872.

[21] D.A. Waldow, M.D. Ediger, Y. Yamaguchi, Y. Matsushita, I. Noda, Viscosity dependence of the local segmental dynamics of anthracene-labeled polystyrene in dilute solution, Macromolecules 24 (1991) 3147–3153.

[22] J. Horinaka, K. Ono, M. Yamamoto, Local chain dynamics of syndiotactic poly(methyl methacrylate) studied by the fluorescence depolarization method, Polym. J. 27 (1995) 429–435.