# Gel Formation of K-gellan in the Presence of Sodium Dodecyl Sulfate

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Effect of sodium dodecyl sulfate (SDS) on the sol-gel transition of potassium type gellan (K-gellan) was investigated by circular dichroism (CD), differential scanning calorimetry (DSC) and dynamic viscoelastic measurements. CD and DSC results showed that SDS stabilized helices thermally as observed in the case of addition of salts to gellan. The addition of SDS lowered the storage modulus of the gellan gels and thermal hysteresis became less pronounced, which was not observed in gellan in the presence of salts. It was suggested that gel formation of K-gellan was prevented by anions in SDS, i.e. the anionic surfactant affects properties of the anionic polysaccharide.

Key words: gellan gum, SDS, sol-gel transition, helix-coil transition, viscoelasticity

## 1. INTRODUCTION

Thermoreversible gels of helix-forming polysaccharides have been used for a long time especially in food and biomedical industries. In food industries, texture of food products has been controlled using the gels and this has been done empirically due to the lack of basic knowledge about formation and properties of the gels. As thermoreversible gels are being studied in polymer science, these studies are becoming helpful to predict gelation and gel properties more precisely [1,2].

It has been considered that gel formation of most of helix-forming polysaccharides is induced by helix formation and aggregation between helices. The sol-gel transition of anionic polysaccharides, for instance κ-carrageenan and gellan, has been studied well in the presence of cations since cations promote their helix formation and aggregation strongly [3,4]. When both helix formation and aggregation were promoted simultaneously, it is difficult to understand the role of helices and aggregates in the gels. Converting κ-carrageenan or gellan to tetramethylammonium (TMA) salts is a conventional method to minimize aggregation and study a helix-coil transition, in which bulky TMA ions do not allow the helices to aggregate [5,6]. Another method to prevent aggregation of helices of κ-carrageenan is the addition of iodide ions. Grasdalen and Smidsröd reported that the gel formation of κ-carrageenan was prevented strongly by the addition of iodide ions whereas helix formation was promoted [7] and hence it was concluded that iodide ions prevent aggregation between

helices. Adding NaI to κ-carrageenan prevented the aggregation of helices and developed the interpretation of the role of helices and aggregates of helices [8-10].

Gellan gum is a microbial polysaccharide and consists of a tetra-saccharide repeating unit, β-D-glucose, β-D-glucuronic acid, β-D-glucose, and α-L-rhamnose [11,12]. Gellan gum forms thermoreversible gels that are transparent and resistant to heat and pH under appropriate conditions [13]. Fifteen laboratories with different methodologies in the Research Group on Polymer Gels affiliated to the Japan Society of Polymer Science have been studying gellan gels as a model of thermoreversible gels consisting helices since 1990 [14-16]. As was done for κ-carrageenan [8-10], it is desirable to prepare a system in which helices exist without aggregates for elucidating gelation mechanism of gellan. If the additives have a large volume or they show some specific interaction with gellan, some of them could inhibit the aggregation of gellan molecules.

Sodium dodecyl sulfate (SDS) is a widely used surfactant and has a large anion. It is expected that the large anions minimize aggregation between helices. SDS forms micelles above the critical micelle concentration (~8mM) and this also might influence aggregation between helices. In addition, investigating viscoelastic properties of gellan in the presence of SDS is important not only as a study of polymer-surfactant interaction but also for applying gellan to cosmetic and pharmaceutical industries. In the present study, effect of SDS on the

sol-gel transition of gellan was investigated using circular dichroism (CD), differential scanning calorimetry (DSC) and dynamic viscoelastic measurements.

## 2. EXPERIMENTAL

Gellan of potassium salt form (K-gellan) was kindly supplied by San Ei Gen FFI Ltd. (Japan). The content of K, Na, Ca, and Mg in the gellan was 2.08%, 0.19%, 0.51%, and 0.15%, respectively. SDS of reagent grade was purchased from Sigma Aldrich Japan Ltd. (Japan). Rheostress1 was kindly lent to us by Haake Ltd. (Germany) and used to measure the storage and loss shear modulus $G'$ and $G''$. A double gap cylinder was used and a serrated cylinder was used only when fatal artifacts were observed probably due to slippage [17]. Jasco-820 spectropolarimeter of Nihon Bunko Ltd. (Japan) was used to measure the ellipticity at 202nm using a cell, the path length of which was 0.2mm.

The gellan powder was dispersed in distilled water and was stirred at 40°C overnight. The dispersion was heated at 80°C for 1hr and 90°C for 10min before an appropriate amount of SDS was added and then poured to a cup equipped with Rheostress1 or the cell equipped with the spectropolarimeter at 70°C.

## 3. RESULTS AND DISCUSSION

Fig.1 shows temperature dependence of the ellipticity at 202nm, $\theta_{202}$, obtained in CD measurements for the gellan with or without added SDS. $\theta_{202}$ decreased steeply at a characteristic temperature on cooling, which reflects coil-to-helix transition of gellan [18,19]. Tm, the temperature at the maximum of $d\theta_{202}/dT$ (data not shown), shifted to higher temperatures by addition of SDS, suggesting that helices were more thermally stabilized by addition of SDS. This tendency was observed in DSC cooling curves in which helix formation can be detected as an exothermic peak. Fig.1 (b) shows cooling DSC curves of the gellan with or without SDS. The broad exothermic peak was observed around 35°C for the gellan sample in the absence of SDS. The broadness of the peak was due to containing various cations in the present sample [20]. By addition of SDS the single sharp peak appeared and shifted to higher temperatures. Tm obtained in CD measurements agreed well with DSC peak temperatures. DSC results also suggested that helices were stabilized thermally by addition of SDS. Sharp peak was observed and DSC peaks shifted to higher

temperatures previously by addition of salts such as NaCl [3,4], suggesting that effect of SDS on helix formation is similar to that of salts, i.e. cations promoted helix formation by shielding electrostatic repulsion between anionic polysaccharides [3,4].
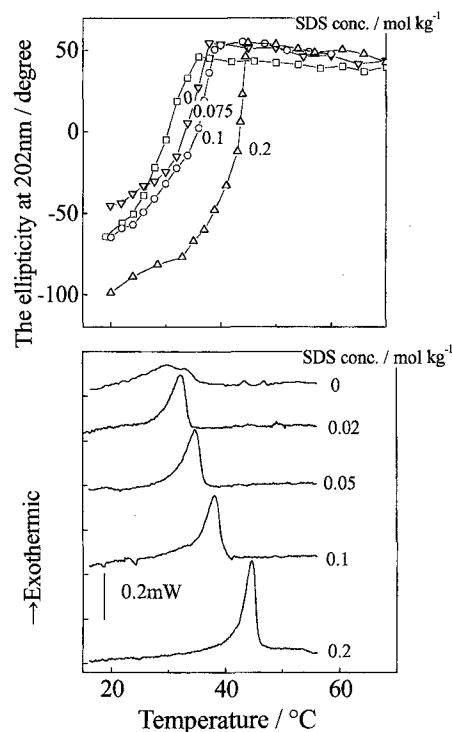


Fig. 1(a) Temperaure dependence of the ellipticity at 202nm $\theta_{202}$ and (b) DSC curves on cooling of 1.6% (w/w) K-gellan with or without added SDS.

Fig.2 shows temperature dependence of the shear storage modulus $G'$ of 1.6% (w/w) K-gellan solutions and 1.6% (w/w) K-gellan with SDS. Since addition of SDS above 0.2mol·kg$^{-1}$ caused phase separation measurements were performed below 0.2mol·kg$^{-1}$ SDS. Gellan sample at this concentration formed strong gels with high elastic modulus. $G'$ increased rapidly around 35°C on cooling and became 11000 Pa at 20°C. This indicates that gelation occurred around 35°C. On subsequent heating $G'$ decreased gradually first and then decreased rapidly around 90°C, indicating that gel melting might occur around 90°C. This large thermal hysteresis on cooling and on heating is a characteristic feature of true gels like agarose gels and it is considered that this thermal hysteresis was due to thermally stabilized network by aggregation between helices [21]. By addition of SDS up to certain amount a strong gel changed to a weak gel. By addition of relatively small amount of SDS $G'$ decreased

remarkably and the degree of thermal hysteresis was lowered as shown in Fig.2 (a). In the presence of $0.02 \text{mol·kg}^{-1}$SDS, $G'$ became the order of 10 Pa and the thermal hysteresis was negligible. This suggests that three-dimensional network formation and aggregation between helices were prevented by addition of $0.02 \text{mol·kg}^{-1}$SDS. The small $G'$ and negligible thermal hysteresis were obtained by addition of $0.02 \text{mol·kg}^{-1}$, $0.05 \text{mol·kg}^{-1}$, and $0.1 \text{mol·kg}^{-1}$SDS in the present study. In the presence of relatively large amount of SDS as shown in Fig.2 (b), $G'$ increased with increasing concentration of SDS and strong gel was obtained in the presence of $0.2 \text{mol·kg}^{-1}$ SDS. $G'$ became 4500 Pa at 20°C and thermal hysteresis was observed. This suggests that a large amount of SDS induced network formation and aggregation between helices. As shown in Fig.1, DSC and CD results suggested that SDS stabilized helices of K-gellan thermally. If SDS stabilizes gellan helices, it is understandable for SDS to promote gel formation and aggregation. However, the value of $G'$ and the degree of thermal hysteresis of gellan gels with SDS, at any SDS concentration, was lower than those of the gellan gels in the absence of SDS. Thus the viscoelastic behaviour of K-gellan in the presence of SDS could not be interpreted without involving effect of anions in SDS. This indicates that the anionic surfactant affects gel formation of the anionic polysaccharide.

Frequency dependence of $G'$ and $G''$ showed that the gellan gels changed to weak gel and viscoelastic fluid by addition of $0.02$-$0.1 \text{mol·kg}^{-1}$SDS as shown in Fig.3. $G'$ and $G''$ of the gellan sample without SDS and with $0.01 \text{mol·kg}^{-1}$ SDS showed "true gel" type behaviour in which $G'$ and $G''$ were almost independent of frequency and $G'$ was 10 times larger than $G''$. $G'$ and $G''$ of the gellan sample with $0.02 \text{mol·kg}^{-1}$ SDS showed "weak gel" type behaviour in which $G'$ and $G''$ slightly depend on frequency keeping $G' > G''$ in the frequency range observed. $G'$ and $G''$ of the gellan sample with $0.05 \text{mol·kg}^{-1}$ SDS showed "semi-dilute solution" type behaviour in which $G' < G''$ at lower frequencies and $G' > G''$ at higher frequencies. $G'$ and $G''$ of the gellan sample with $0.1 \text{mol·kg}^{-1}$ and $0.2 \text{mol·kg}^{-1}$ SDS showed "weak gel" type behaviour and "true gel" type behaviour, respectively. When "weak gel" or "semi-dilute solution" type behaviour was observed, the thermal hysteresis on cooling and on heating was not observed. These tendencies were recognized in κ-carrageenan and NaI systems in which

iodide ion prevents gel formation by preventing aggregation between helices. κ-carrageenan/NaI system formed network that flowed by applying large deformation. In addition, after large deformation this system showed gel like behaviour again.
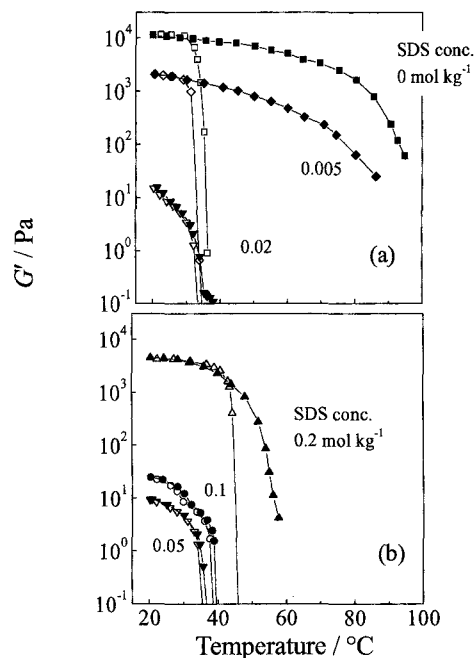


Fig. 2. (a) (b) Temperature dependence of $G'$ of 1.6% (w/w) K-gellan with or without added SDS on cooling (open) and on subsequent heating (closed) at 0.5°C/min. Frequency; 1rad/s, strain; 0.5%.
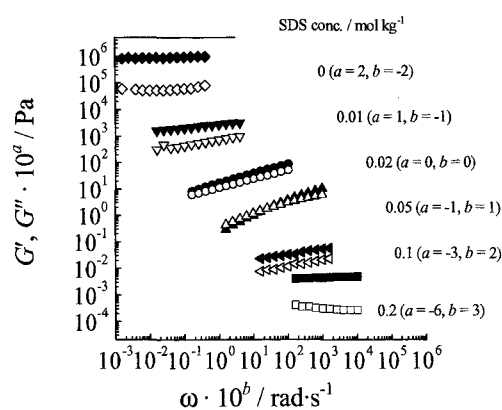


Fig. 3. Frequency dependence of $G'$ (closed) and $G''$ (open) of 1.6% (w/w) K-gellan with or without SDS at 20°C. The data are shifted along the vertical and horizontal axes by shift factor $a$ and $b$, respectively, to avoid overlapping. Strain; 0.5%.

Fig.4 shows $G'$ and $G''$ of the gellan sample with $0.1 \text{mol·kg}^{-1}$ SDS as a function of strain at 20°C. At small deformation $G'$ was larger than $G''$ and frequency

dependence of $G'$ and $G''$ showed a "weak gel" type behaviour. After large deformation measurements the same experiment was repeated. A similar tendency, $G' > G''$ at a small deformation range, was observed in the second- and the third-run. As suggested previously for non-aggregated helices of κ-carrageenan in the presence of NaI, the network of K-gellan with 0.1mol·kg$^{-1}$ SDS is not permanent but capable of flowing [9]. This indicates that this K-gellan network in the presence of SDS is similar to that of κ-carrageenan in the presence of NaI.
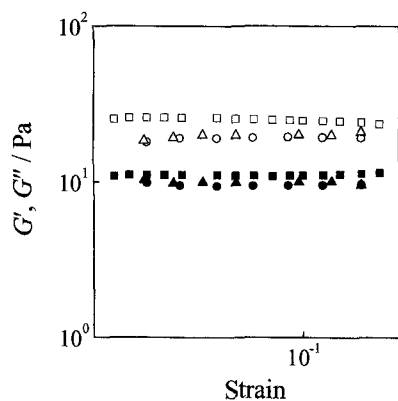


Fig. 4. $G'$ (open) and $G''$ (closed) as a function of strain of 1.6% (w/w) the K-gellan gels in 0.1mol/kg SDS at 20°C. The first (square), the second (triangle) and the third (circle) run were shown. Frequency; 1rad/s.

As suggested in the case of κ-carrageenan/NaI, SDS might prevent gel formation of K-gellan by preventing aggregation since it did not prevent helix formation judging from CD and DSC results. This effect can be achieved if bulky anions in SDS exist around gellan helices by some interactions (e.g. hydrophobic interaction) and do not allow gellan helices to aggregate. However excessive amount of SDS induced strong gel with thermal hysteresis which was not observed in κ-carrageenan/NaI. It is known that addition of cations and sugars promotes gel formation of gellan up to certain amount and excessive amount of cations and sugars decreased the elastic modulus [22,23]. The mechanism of gel formation in the presence of excessive amount of such substances has not been understood well. It is considered that "over conversion" of gellan occurs [23]. It should be further studied using gellan of sodium salt form that shows single sharp peak in DSC curves so that we can neglect effects of other cations in gellan except for Na$^+$.

In conclusion, it was found that SDS, anionic surfactant, affects properties of K-gellan, anionic polysaccharide,

strongly. Helix formation of K-gellan was promoted by addtion of SDS, probably due to cations in SDS whereas gel formation of K-gellan was prevented by addition of SDS, which could not be explained without involving effect of anions in SDS. Viscoelastic fluids and weak gels of K-gellan induced by addition of SDS showed similar behaviour against thermal history and large deformation to those of κ carrageenan induced by addition of NaI in which NaI prevents aggregation between helices and promotes helix formation of κ carrageenan.

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