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## Note

# Indicative evidence for a conformational transition in κ-carrageenan from studies of viscosity-shear rate dependence

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κ-Carrageenan, which consists of alternating disaccharide units of (1→3)-linked β-D-galactose 4-sulfate and (1→4)-linked 3,6-anhydro-α-D-galactose, is well known for its gel-forming property[1–3]. A mechanism of gelation was proposed that involves the formation of a double or a single helix; but whether κ-carrageenan exists as a double or single helix is still under discussion[4,5]. We describe herein the rheological behavior of κ-carrageenan with respect to its association characteristics. This work may offer a new concept for gel formation in κ-carrageenan as compared to the previous models[4,6]

## EXPERIMENTAL

*Materials.* — κ-Carrageenan extracted from *Eucheuma cottonii* was supplied by the Taiyo Kagaku Co., Ltd. It was dissolved in water as a 0.5% solution and filtered through Celite 545 which had been treated with boiling 3M HCl for 30 min, and washed with distilled water until the pH was 6.5. In the presence of 0.5% KCl, ethanol (2 vol.) was added to the filtrate, and the precipitate was dried *in vacuo*. Purified κ-carrageenan was redissolved in water again, and de-ionized by passage through a column of Amberlite IR-120 ($H^+$) cation-exchange resin, and then neutralized with 100mM KOH, NaOH, or $Ca(OH)_2$. Ethanol (2 vol.) was added to the filtrate in the presence of 0.5% KCl, NaCl, or $Ca(Cl)_2$, and the precipitate was dried *in vacuo*. The identity of the polysaccharide as κ-carrageenan was controlled by determination of the i.r. spectra, recorded with a spectrophotometer IR 440 (Shimadzu Seisakusho Co., Ltd.) and of the specific rotation measured at 589 nm with an automatic digital polarimeter DIP-180 (Japan Spectroscopic Co., Ltd.) for solutions of 0.5% in water.

*Viscosity and dynamic viscoelasticity measurements.* — Viscosity at different shear rates (1.188–76.021 $s^{-1}$) and dynamic viscoelasticity at steady angular velocity (3.768 rad/s) were determined with a rheogoniometer consisting of a coaxial cylinder (1.8-cm diam.) with rotating outer cylinder (2.2-cm diam.), 6.0-cm long (IR-

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103, Iwamoto Seisakusho Co., Ltd.). The temperature of the sample was controlled by circulating oil from a thermo-cool (LCH-130F, Toyo Co., Ltd.) over the temperature range of 0–30° and raised at a rate of 1°/min by steps. Shear rate ($D$), shear stress ($S$), and apparent viscosity ($\eta$) were calculated with the equation of Margules[7]. Dynamic viscosity ($\eta'$) and elasticity($G'$) were calculated by a modification of Markovitz's equation[8]. The loss tangent was calculated from the relationship, $\tan \delta = G''/G'$, where $G'' = \omega\eta'$ is the loss modulus, and $\omega$ is the angular velocity of the outer cylinder.

## RESULTS

I.r. spectra, obtained from a dispersion of the polysaccharide in KBr discs, showed the characteristic absorption band at 850 $\text{cm}^{-1}$ which was attributed to the D-galactose 4-sulfate residue[9]. The content of sulfate ester was estimated at 16.2% by precipitation as barium sulfate[10]. The specific rotation of κ-carrageenan (potassium salt), measured with an automatic digital polarimeter for a 0.5% solution in water, was +73.2, +54.8, and +46.8°, at temperatures of 5, 30, and 60°, respectively; as compared to the optical rotation of the native, sodium, and calcium form, these values agreed with those of the native form.

The flow curve, at 25°, of κ-carrageenan (potassium salt) at various concentrations is shown in Fig. 1. For concentrations of 0.5 and 0.8%, the flow curve approximated Newtonian behavior, for 1.0% concentration it approximated pseudoplastic behavior, and for 1.5 and 2.0% concentrations plastic behavior. The yield value for the two last-named concentrations was estimated to be 4 and 10 dyne/$\text{cm}^2$, respectively. The native form of κ-carrageenan showed a flow-behavior similar to that of the potassium salt, but the sodium salt showed Newtonian behavior even at a 2.0% concentration. At 20°, gelation occurred for both the native and potassium form of κ-carrageenan at a concentration of 2.0%, but not for the sodium and calcium salts. Thus, the flow characteristic of κ-carrageenan varies as

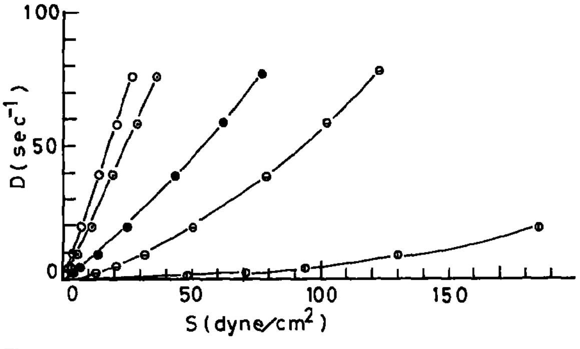


Fig. 1. Flow curves of the potassium salt of κ-carrageenan, at various concentrations and 25°. Concentration: (○) 0.5, (⊙) 0.8, (●) 1.0, (⊖) 1.5, and (⦶) 2.0%.

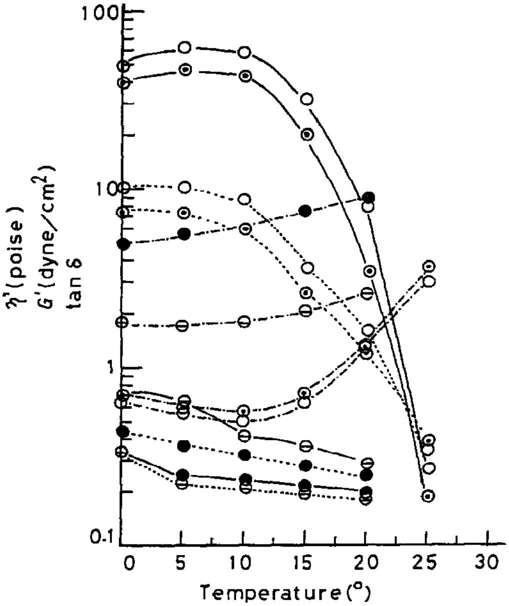


Fig. 2. Effects of temperature on the dynamic viscoelasticity, at 3.768 rad/s, for a 0.8% solution of κ-carrageenan in various cation forms. Cation form: (○) native, (⊙) K form, (●), Na form, and (⊖) Ca form; (····) dynamic viscosity, (——) dynamic modulus, and (-·-·-) tan δ.

the concentration increases. These results suggest that the conformation of κ-carrageenan in aqueous solution may adopt a rod-like structure[11,12] at 25°.

Gelation occurred for both the native and potassium form of κ-carrageenan at a concentration >0.8% at low temperature (0°), but not for the sodium form, even at a concentration of 2.0%. At a concentration of 0.8% (see Fig. 2), the dynamic modulus of both native and potassium forms increased with the increase in temperature, showing a maximum value at 10°, and then it decreased rapidly. This increase of the dynamic modulus was similar to that of xanthan, the dynamic viscoelasticity of which showed a sigmoid curve[13], and has been attributed to the breakdown and temporary formation of intra- and inter-molecular association[14]. The tan δ value of κ-carrageenan (see Fig. 2) stayed constant during increase of the temperature until 15°, then it increased. This behavior also agreed with that of native xanthan[13] and suggests the co-existence, in κ-carrageenan, of intra- and inter-molecular association. The dynamic viscoelasticity of the sodium and calcium salts showed values lower than those of the native and potassium forms, and which decreased with increase of the temperature.

As shown in Fig. 3, a very large increase of the dynamic viscoelasticity was observed on the addition of a 0.1% solution of potassium chloride to a 0.2% solution of the native and potasium forms of κ-carrageenan at low temperature. The tendency of the curves showing the dynamic modulus for an increase in temperature

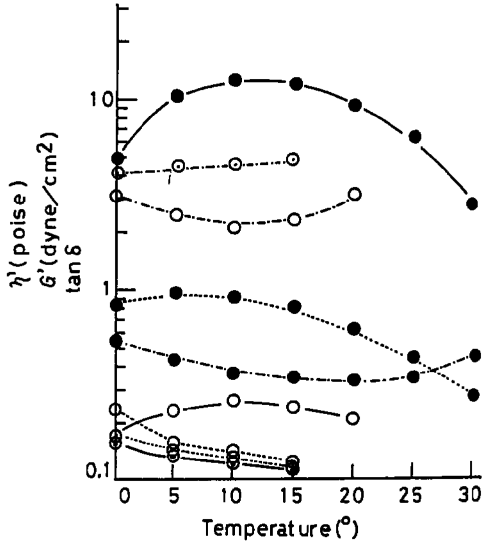
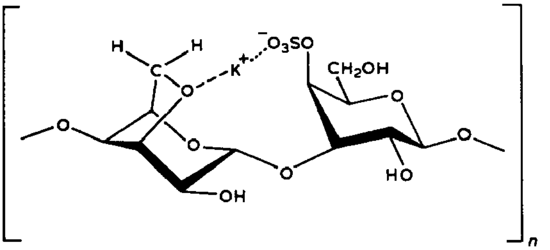


Fig. 3. Effects of temperature on the dynamic viscoelasticity, at 3.768 rad/s, for a 0.2% solution of the potassium salt of κ-carrageenan after the addition of 0.1% salts: (○) κ-carrageenan alone, (●) with addition of KCl, and (⊙) with addition of NaCl; (····) dynamic viscosity, (——) dynamic modulus, and (-·-·-·) tan δ.

is in agreement with that of the native and potassium forms without addition of potassium chloride (Fig. 2), which suggests that intramolecular association also took place in the presence of added potassium chloride in addition to intermolecular association, but not in the presence of sodium or calcium chloride. At 0°, gelation was observed in the acid and alkaline range after reaching pH values of 2.2 and 11.3 by addition of 50mM ~2M hydrochloric acid, or potassium, sodium, or calcium hydroxide. Upon addition of 4.0M urea, the dynamic viscoelasticity was lower than that of κ-carrageenan alone.

## DISCUSSION

It has been suggested, in the Results section, that the native and potassium forms of κ-carrageenan molecules involve both intra- and inter-molecular association at low temperature. κ-Carrageenan is a cation-selective binding polymer[3–5], which gels in the presence of large-site cations, *i.e.*, $K^+$, $Rb^+$, and $Cs^+$, but does not do so in the presence of small cations, *i.e.*, $Na^+$ and $Li^+$. This characteristic behavior was confirmed for the potassium and sodium salts of κ-carrageenan in the present study. The cations having a negative Jones–Dole B coefficient of viscosity are structure disordering and show negative hydration, whereas the cations having a positive coefficient are structure ordering and show positive hydration[15,16]. The $K^+$ ion has a negative B coefficient whereas the $Na^+$ ion has a positive one[15]. Furthermore, the anhydrogalactose residues affect the hydrophobic character of the



Scheme 1. Possible mode of intramolecular, cation-selective bridge in the κ-carrageenan molecule in aqueous solution at low temperature: (····) ionic bonding and (----) electrostatic force of attraction. The large cations ($K^+$, $Rb^+$, and $Cs^+$) are able to build up the bridge, but the small cations ($Na^+$ or $Li^+$) are not.

polymer by controlling its solubility. At low temperature, in the presence of ionic bonding between the sulfate group and a large cation, the adjacent anhydro oxygen atom bond would be attracted to the large cation by the electrostatic force. On this basis, we propose an intramolecular, cation-selective bridge for the κ-carrageenan molecule (see Scheme 1). The bridge may be built at low temperature owing to the decrease in kinetic energy and Brownian motion of the molecular chain and solvent, but dissociation takes place at room temperature (25°) owing to an increase in kinetic energy and Brownian motion. The bridge is likely to be established in view of the flexibility of the α-D-(1→3) linkage between the anhydro-D-galactose and the D-galactose 4-sulfate residues, and corresponds to a rod-like[11,12] and less-expanded structure[17]. The intramolecular bridge does not take place in the presence of $Na^+$ ion in spite of the ionic bonding still being present at low temperature, because the radius of the ion is too small for an association with the ring oxygen group and the presence of too much hydration may prevent an electrostatic force of attraction being involved in the bridge.

On the basis of the intramolecular cation-bridge proposed, a model of intermolecular association of the κ-carrageenan molecules was developed. As the kinetic energy and Brownian motion of the molecules and solvent decreases at low temperature, many intramolecular cation-bridges serve to keep rigid the chains, which results in an intermolecular association. This suggests that the ring oxygen groups play the same role in the intermolecular association as do the sulfate groups. This hypothesis is in agreement with the increase of intermolecular association by the addition, at low temperature, of the $K^+$ ion, because this ion is a univalent cation.

An intermolecular, cation-selective bridge has been proposed by Bayley[18] before the polysaccharide structure was known. However, the mode of association proposed did not take into consideration the role of the intramolecular bridge that we are proposing.

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