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Graphical abstract



Effect of monovalent cations on calcium-induced assemblies of kappa carrageenan

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1 Abstract

The effect of Na^+ , K^+ and Ca^{2+} cations on the thermal stability and aggregation of kappa 2 3 carrageenan double helices has been explored by differential scanning calorimetry (DSC). 4 Previous studies have shown that kappa carrageenan helices bind K⁺ cations, but not Na⁺. 5 The kappa carrageenan used in this work was therefore in the Na⁺ salt form, to avoid 6 complications from site-bound counterions to the polymer, and was studied at a fixed concentration of 1.0 wt % (~25 mN w.r.t. sulfate groups). Na⁺, K⁺ and Ca²⁺ cations were 7 8 added as chloride salts. Values of peak-maximum temperature (T_{max}) in DSC cooling and 9 heating scans (0.5°C/min) increased progressively with increasing salt concentration, following the order Na⁺< Ca²⁺< K⁺, but greatest thermal hysteresis was seen with Ca²⁺. 10 Our proposed interpretation is that Ca²⁺ cations "cement" the carrageenan helices together by 11 12 binding directly between them, giving greater thermal stability, and thus greater hysteresis, than K⁺ cations which act indirectly by suppressing charge. On progressive addition of NaCl 13 or KCl to solutions incorporating Ca²⁺ at concentrations of 5 mM or 12.5 mM (stoichiometric 14 15 equivalence) the values of T_{max} moved asymptotically towards those seen for the same 16 concentrations of the monovalent cations in the absence of calcium, suggesting progressive displacement of site-bound Ca²⁺. Thus T_{max} for the order-disorder transition was increased by 17 18 KCl but reduced by NaCl, with the strange consequence that addition of NaCl lowered the 19 transition temperature rather than raising it.

20 Keywords

21 Kappa carrageenan; Differential scanning calorimetry; Calcium ions; Cation binding

23 1. Introduction

Kappa carrageenan is a structural polysaccharide from numerous species of red seaweed
(Rhodophyta) and is used extensively as a gelling agent, particularly in food products
(Glickman, 1983; Stanley, 1990; Therkelsen, 1993).

27 It is one of a family of sulfated galactans (Painter, 1983) with primary structures based on a linear disaccharide repeating sequence of alternating 1,3 and 1,4-linked residues. The 28 29 other gelling carrageenan used commercially is iota. In both iota and kappa carrageenan the 30 1,4-linked residues occur predominantly as the 3,6-anhydride (Fig. 1), and the 1,3-linked 31 residues have a sulfate group at C(4). In nature, the anhydride bridge is absent in a significant 32 proportion of the 1.4-linked residues, but in commercial production structural regularity is 33 normally enhanced by treatment with alkali (Smidsrød, Larsen, Penas & Haug, 1967; 34 Stanley, 1963), giving structures close to the idealised repeating sequence in Fig. 1. Unlike 35 kappa carrageenan, iota has an additional sulfate substituent at C(2) of the 1,4-linked residue 36 (Anderson, Dolan & Rees, 1973). Thus iota carrageenan has two negative charges per 37 disaccharide, whereas kappa has only one.

This difference in pattern of sulfation causes profound differences in gelation behaviour. (Picullel, 1995). Iota carrageenan gels are formed on cooling by conversion of disordered coils to 3-fold double helices (Arnott, Scott, Rees & McNab, 1974) which constitute the junction zones of the gel network (Rees, 1970). The coil-helix and accompanying sol-gel transitions are reversed on heating, with little, if any, thermal hysteresis.

43 Kappa carrageenan has a double helix structure similar to that of iota (Anderson, 44 Campbell, Harding, Rees & Samuel, 1969; Millane, Chandrasekaran, Arnott & Dea, 1988). 45 Again, gels form on cooling and melt on heating, but with substantial thermal hysteresis 46 (in marked contrast to the sol-gel and gel-sol transitions of iota). Hysteresis is attributed to 47 cation-induced aggregation of the kappa carrageenan double helices, with the aggregates 48 surviving to higher temperatures than those at which individual helices are formed on cooling 49 (Morris & Norton, 1983; Morris, Rees & Robinson, 1980). Some limited aggregation of 50 helices, with associated slight thermal hysteresis, may also occur for iota carrageenan in the 51 presence of high concentrations of Ca²⁺ cations (Goycoolea, Morris & Gidley, 1995; Tari, 52 Kara & Pekcan, 2010).

It has been demonstrated by NMR (Belton, Morris & Tanner,1986; Grasdalen & Smidsrød, 1981; Piculell, Nilsson & Ström, 1989) that, unlike Li⁺ and Na⁺, K⁺ (and larger Group I cations) bind to kappa carrageenan double helices. This reduces the net negative charge on the helices, and promotes helix–helix aggregation by suppressing electrostatic repulsion. Aggregation increases gel strength by giving an additional mechanism of crosslinking (Viebke, Piculell & Nilsson, 1994), and commercial kappa carrageenan is therefore normally produced and supplied with K⁺ as the predominant cation.

61 The interaction of kappa carrageenan with Ca²⁺ is less well understood. On addition of increasing concentrations of K⁺ to 1.0 wt % solutions of kappa carrageenan in the Na⁺ salt 62 63 form (to avoid complications from site-binding of counterions to the polymer), Doyle, 64 Giannouli, Philp & Morris (2002) observed a large, monotonic increase in gel strength. With Ca²⁺ cations, by contrast, gel strength passed through a maximum at stoichiometric 65 equivalence to the sulfate groups of the carrageenan. Maximum gel strength at stoichiometric 66 67 equivalence of Ca^{2+} to the negative charge of the polymer has been observed subsequently (Doyle, Giannouli, Richardson & Morris, 2018) for other concentrations of kappa carrageen 68 (in the range 0.5 - 3.0 wt %), suggesting that Ca^{2+} cations promote aggregation of the double 69 70 helices by site-binding between them (rather than by binding to individual helices, as happens 71 with K^+).

In the present work, we have used differential scanning calorimetry to explore the effect of monovalent cations (K^+ and Na^+) on the stability of Ca^{2+} -induced aggregates of kappa carrageenan.

75 **2. Materials and methods**

Kappa carrageenan in the Na⁺ salt form was kindly supplied by Quest International Ireland
Ltd., Carrigaline, Co. Cork, Ireland. It is an alkali-modified extract from *Euchema cottonii*,
identical to the material used by Doyle et al. (2002) and Doyle et al. (2018). NaCl, KCl and
CaCl₂ were AnalaR grade from BDH. Distilled deionised water was used throughout.

Differential scanning calorimetry (DSC) measurements were made at a fixed carrageenan concentration of 1.0 wt % (~25 mM w.r.t. sulfate groups) using a Seteram DSC III scanning microcalorimeter at heating and cooling rates of 0.5°C/min. Water was used as thermal reference, and sample and reference pans were balanced to within 0.05 mg (typical loading ~850 mg).

Carrageenan was dissolved at 2.0 wt % by mechanical stirring at 80°C. Solutions of NaCl,
KCl and/or CaCl₂ were also prepared at twice the required concentration and mixed in equal
volumes with 2.0 wt % kappa carrageenan at 80°C, to give mixtures containing 1.0 wt %
polymer in the presence of the appropriate concentration of salt(s).

To take account of non-specific screening of electrostatic repulsion between carrageenan
helices, comparisons were made at equivalent values of ionic strength (with 1 mM CaCl₂
having the same ionic strength as 3 mM NaCl or KCl).

92 **3. Results**

93 Thermal hysteresis between the DSC exotherm arising from the disorder-order transition on 94 cooling and the endotherm from the order-disorder transition on heating is illustrated in Fig. 2 95 for 1.0 wt % Na⁺ kappa carrageenan in the presence of 100 mM KCl. The same general 96 pattern of a sharp exotherm and somewhat broader endotherm was observed for all 97 carrageenan-salt mixtures studied, but with large differences in the position of the DSC peaks 98 on varying concentration and type of salt.

Disorder-order and order-disorder transition temperatures can be conveniently characterised by the temperature of maximum (absolute) heat flow in DSC (i.e. the temperature, T_{max} , at the top of the exotherms and bottom of the endotherms). As shown in Fig. 3, the effectiveness of increasing concentrations of the three salts studied in raising T_{max} values for 1.0 wt % Na⁺ kappa carrageenan followed the order NaCl < CaCl₂ < KCl for both the disorder-order transition on cooling (Fig. 3a) and the order-disorder transition on heating (Fig. 3b).

105 However, the extent of thermal hysteresis (Fig. 4) between the ordering and disordering 106 processes followed a different order: $NaCl < KCl < CaCl_2$. Greater thermal hysteresis with CaCl₂ than with KCl is consistent with the proposal (Doyle et al., 2002) that Ca^{2+} cations 107 "cement" kappa carrageenan double helices together by binding between them in an 108 109 arrangement analogous to the "egg box" binding (Grant, Morris, Rees, Smith & Thom, 1973) of Ca²⁺ between polyuronate sequences in calcium alginate and calcium pectinate gels, 110 111 whereas K⁺ acts indirectly by binding to individual helices and thus suppressing electrostatic 112 repulsion.

113 To explore the stability of Ca^{2+} -induced aggregates of kappa carrageenan double helices 114 the effect of adding monovalent cations (Na⁺ and K⁺) was investigated. Two different 115 concentrations of Ca^{2+} were used in mixtures with 1.0 wt % Na⁺ kappa carrageenan: 12.5 mM 116 (stoichiometric) and 5 mM, and increasing concentrations of NaCl or KCl were added.

117 As shown in Fig. 5, as the concentration of KCl was raised the observed values of T_{max} for 118 the disorder–order transition on cooling (Fig. 5a) and the order–disorder transition on heating

119 (Fig. 5b) increased from those observed with 12.5 or 5 mM Ca^{2+} alone towards the higher

120 values found (Fig. 3) with KCl, suggesting displacement of bound Ca^{2+} cations.

When the same experiment was repeated (Fig. 6) using NaCl instead of KCl, addition of high concentrations of NaCl was found to cause a slight increase (Fig. 7a) in the temperature of the disorder-order transition on cooling (Fig. 6a), which can be readily explained by non-specific screening of electrostatic repulsion between the charged polymer coils, thus facilitating intermolecular association into double helices.

126 In the heating direction (Fig. 6b), however, increasing concentrations of NaCl caused a 127 progressive reduction in T_{max} for the disorder-disorder transition, towards the lower 128 values observed (Fig. 3) for NaCl alone. In consequence, addition of high concentrations 129 of NaCl had the surprising effect of *lowering* the transition temperature (Fig. 7b), rather than 130 giving the increase in T_{max} that would be expected from the general behaviour of charged 131 polysaccharides.

132 **4. Discussion and conclusions**

The obvious interpretation of the results presented in Figs. 5 - 7 is that progressive addition of KCl or NaCl causes progressive displacement of Ca^{2+} cations bound between kappa carrageenan double helices, with the overall behaviour then approaching that observed (Fig. 3) for the same concentrations of the monovalent cations in the absence of Ca^{2+} . The outcome is an increase in thermal stability of the helix–helix aggregates on addition of KCl and a reduction with NaCl, which leads to the unusual (perhaps unprecedented) observation that addition of NaCl lowers the temperature of the order–disorder transition (Fig. 7b).

The ability of monovalent cations to displace Ca^{2+} is, in itself, further evidence of site-binding of calcium ions to kappa carrageenan double helices (if they weren't bound they could not be displaced), and reinforces the conclusion from greater thermal hysteresis with Ca^{2+} than with K^+ (Fig. 4) and maximum in gel strength at stoichiometric equivalence of Ca^{2+} to sulfate groups of the polymer (Doyle et al., 2002) that calcium ions bind *between* the double helices, rather than to individual helices.

147 The ability of monovalent salts to displace Ca²⁺ cations bound to anionic polysaccharides 148 has been demonstrated previously for calcium alginate (Morris, Rees, Thom & Boyd, 1978) 149 and calcium pectinate (Morris, Powell, Gidley & Rees, 1982). Both of these form "egg box" 150 structures (Grant et al., 1973) in which calcium ions are sandwiched in site-bound arrays 151 between 2-fold (zig-zag) sequences of polyguluronate (from alginate) or polygalacturonate 152 from pectin. On addition of increasing concentrations of monovalent cations (Na⁺ or Me₄N⁺), 153 the amount of bound Ca²⁺ dropped from around stoichiometric equivalence to the carboxylate 154 groups of the polyuronate sequences to 50% stoichiometric, indicating a highly stable dimeric 155 structure with a single array of calcium ions bound between two polysaccharide chains, with 156 only the inner faces of the 2-fold ordered structures participating in binding.

157 Displacement of Ca^{2+} cations bound between (but not within) "egg box" dimers suggests 158 a parallel with similar displacement from between the dimers (double helices) of kappa 159 carrageenan.

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165 **References**

- 166 Anderson, N.S., Campbell, J.W., Harding, M.M., Rees, D.A., & Samuel, J.W.B. (1969).
- 167 X-ray diffraction studies of polysaccharide sulphates: double helix models for κ and ι -168 carrageenans. *Journal of Molecular Biology*, 45, 85-99.
- 169 Anderson, N.S., Dolan, T.C.S., & Rees, D.A. (1973). Carrageenans. Part VII. Polysaccharides
- from *Eucheuma spinosum* and *Eucheuma cottonii*. The covalent structure of 1-carrageenan.
- 171 Journal of the Chemical Society, Perkin Transactions I, 2173-2176.
- Arnott, S., Scott, W.E., Rees, D.A., & McNab, C.G.A. (1974). 1-Carrageenan: molecular
- structure and packing of polysaccharide double helices in oriented fibres of divalent cation
 salts. *Journal of Molecular Biology*, *90*, 253-267.
- 175 Belton, P.S., Morris, V.J., & Tanner, S.F. (1986). ³⁹K and ⁸⁷Rb NMR study of chemical shift
- 176 effects in ι and κ -carrageenan gels. *Macromolecules*, 19, 1618-1621.
- 177 Doyle, J., Giannouli, P., Philp, K., & Morris, E.R. (2002). Effect of K⁺ and Ca²⁺
- 178 cations on gelation of κ -carrageenan. In P.A. Williams & G.O. Phillips (Eds.),
- 179 *Gums and stabilisers for the food industry 11* (pp. 158-164). Cambridge, UK:
- 180 Royal Society of Chemistry.
- 181 Doyle, J., Giannouli, P., Richardson, R.K., & Morris, E.R. (2018). Site binding
- 182 of cations between double helices in Ca²⁺-induced gelation of κ -carrageenan.
- 183 *Food Hydrocolloids*, in preparation.
- 184 Glicksman, M. (1983). Red seaweed extracts (agar, carrageenans, furcellaran).
- In M. Glicksman (Ed), *Food Hydrocolloids*, Vol. 2 (pp. 73-113). Boca Raton, FL:
- 186 CRC Press.
- 187 Goycoolea, F.M., Morris, E.R., & Gidley, M.J. (1995). Screening for synergistic interactions
- in dilute polysaccharide solutions. *Carbohydrate Polymers*, 28, 351-358.
- 189 Grant, G.T., Morris, E.R., Rees, D.A., Smith, P.J.C., & Thom, D. (1973). Biological
- 190 interactions between polysaccharides and divalent cations: the egg-box model.
- 191 *FEBS Letters*, *32*, 195-198.
- 192 Grasdalen, H., & Smidsrød, O. (1981). ¹³³Cs NMR in the sol–gel states of aqueous carrageenan.
- 193 Selective site binding of cesium and potassium ions in κ-carrageenan gels. *Macromolecules*,
- *194 14*, 229-231.

- 195 Millane, R.P., Chandrasekaran, R., Arnott, S., & Dea, I.C.M. (1988).
- 196 The molecular structure of kappa-carrageenan and comparison with iota carrageenan.
- 197 Carbohydrate Research, 182, 1-17.
- 198 Morris, E.R., & Norton, I.T. (1983). Polysaccharide aggregation in solutions and gels.
- 199 In E. Wyn-Jones & J. Gormally (Eds.), Aggregation processes in solution,
- 200 (pp. 549-593). Amsterdam: Elsevier.
- 201 Morris, E.R., Powell, D.A., Gidley, M.J., & Rees, D.A. (1982). Conformations and
- interactions of pectins. I. Polymorphism between gel and solid states of calcium
 polygalacturonate. *Journal of Molecular Biology*, *155*, 507-516.
- Morris, E.R., Rees, D.A., & Robinson, G. (1980). Cation-specific aggregation of carrageenan
 helices: domain model of polymer gel structure. *Journal of Molecular Biology*, *138*, 349-362.
- 206 Morris, E.R., Rees, D.A., Thom, D., & Boyd, J. (1978). Chiroptical and stoichiometric
- 207 evidence of a specific, primary dimerisation process in alginate gelation. *Carbohydrate*208 *Research*, 66, 145-154.
- Painter, T.J. (1983). Algal polysaccharides. In G.O. Aspinall (Ed.), *The polysaccharides*, Vol. 2
 (pp. 195-285). New York: Academic Press.
- Piculell, L. (1995). Gelling carrageenans. In A.M. Stephen (Ed.), *Food polysaccharides and their applications* (pp. 205-244). New York: Marcel Dekker.
- 213 Piculell, L., Nilsson, S., & Ström, P. (1989). On the specificity of the binding of cations to
- 214 carrageenans: counterion N.M.R. spectroscopy in mixed carrageenan systems Carbohydrate
- 215 *Research*, 188, 121-135.
- 216 Rees, D.A. (1970). A double helix structure in food. *Science Journal*, *6*, 47-51.
- 217 Smidsrød, O., Larsen, B., Pernas, A.J., & Haug, A. (1967). The effect of alkali treatment on
- 218 the chemical heterogeneity and physical properties of some carrageenans. *Acta Chemica*
- 219 *Scandinavica*, *21*, 2585-2598.
- 220 Stanley, N.F. (1963). Process for treating a polysaccharide of seaweeds of
- the Gigartinaceae and Solieriaceae families. US Patent 3 094 517.
- 222 Stanley, N.F. (1990). Carrageenans. In P. Harris (Ed.), Food gels (pp. 79-119).
- 223 Barking, UK: Elsevier Science Publishers.

- 224 Tari, Ö, Kara, S., & Pekcan, Ö (2010). Thermal phase transitions of iota carrageenan in CaCl₂
- solutions: A fluorescence study. *Journal of Macromolecular Science*, *Part B: Physics*, *50*,
 306-318.
- 227 Therkelsen, G.H. (1993). Carrageenan. In R.L. Whistler & J.N. BeMiller (Eds.), Industrial
- 228 gums, Third Edition (pp. 145-180). San Diego, CA: Academic Press.
- 229 Viebke, C., Piculell, L., & Nilsson, S. (1994). On the mechanism of gelation of
- helix-forming biopolymers. *Macromolecules*, 27, 4160-4166.

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232 Legends to figures

Fig. 1. Idealised disaccharide repeating structure of gelling carrageenans. R = H in kappa and SO₃⁻ in iota.

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Fig. 2. DSC traces recorded on cooling and heating at 0.5°C/min for 1.0 wt % Na⁺ kappa
carrageenan with 100 mM added KCl.

239

Fig. 3. Effect of ionic strength on peak-maximum temperature (T_{max}) for 1.0 wt % Na⁺ kappa carrageenan on (a) cooling and (b) heating in the presence of increasing concentrations of NaCl (\blacktriangle), KCl (\blacksquare) or CaCl₂ (\bullet).

243

Fig. 4. Effect of ionic strength on the extent of thermal hysteresis between DSC cooling and heating scans (Fig. 3) for 1.0 wt % Na⁺ kappa carrageenan in the presence of increasing concentrations of NaCl (\blacktriangle), KCl (\blacksquare) or CaCl₂ (\bullet).

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Fig. 5. Variation of peak-maximum temperature with ionic strength on addition of increasing concentrations of KCl to 1.0 wt % Na⁺ kappa carrageenan in mixtures with 5 mM (\bullet) or 12.5 mM (\blacktriangle) CaCl₂ on (a) cooling and (b) heating The corresponding values from addition of KCl (\Box) or CaCl₂ (O) alone (Fig. 3) are shown for comparison.

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Fig. 6. Variation of peak-maximum temperature with ionic strength on addition of increasing concentrations of NaCl to 1.0 wt % Na⁺ kappa carrageenan in mixtures with 5 mM (\bullet) or 12.5 mM (\blacktriangle) CaCl₂ on (a) cooling and (b) heating The corresponding values from addition of NCl (Δ) or CaCl₂ (O) alone (Fig. 3) are shown for comparison.

257

Fig. 7. DSC traces recorded on (a) cooling and (b) heating for 1.0 wt % Na⁺ kappa carrageenan with 12.5 mM CaCl₂ in the presence (solid line) or absence (dashed line) of 100 mM NaCl.



Fig. 1. Idealised disaccharide repeating structure of gelling carrageenans. R = H in kappa and SO₃⁻ in iota.



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Fig. 7. DSC traces recorded on (a) cooling and (b) heating for 1.0 wt % Na⁺ kappa carrageenan with 12.5 mM $CaCl_2$ in the presence (solid line) or absence (dashed line) of 100 mM NaCl.

Highlights

- Site-binding of calcium cations between kappa carrageenan double helices
- Consequent large hysteresis between ordering and disordering transitions in DSC
- Bound Ca²⁺ displaced progressively by increasing concentrations of NaCl or KCl
- KCl moves order-disorder transition towards higher temperatures seen with K⁺ alone
- High concentrations of NaCl lower transition temperature rather than raising it

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