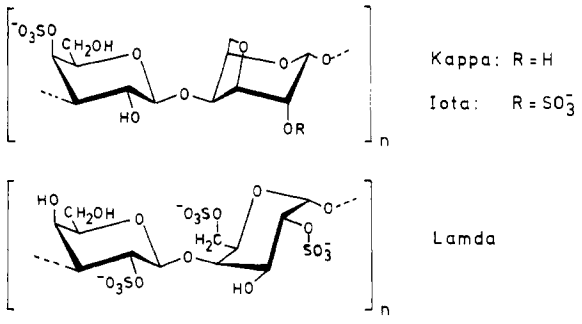
^{133}Cs NMR in the Sol–Gel States of Aqueous Carrageenan. Selective Site Binding of Cesium and Potassium Ions in κ-Carrageenan Gels

The carrageenans are sulfated D-galactans extracted from certain marine red algae and characterized by alternating α(1→3),β(1→4)-linked structures. The 4-linked units are sometimes in the 3,6-anhydro form. Differences in degree of sulfation and 3,6-anhydrogalactose content probably exist both among and regionally within molecules in most extracts. Those from *Eucheuma cottonii*, *Eucheuma spinosum*, and *Gigartina acicularis*, however, appear to approach the idealized structures depicted in formula I with the names "κ-", "ι-", and "λ-carrageenan", respectively.[1-5] Their physical properties, particularly the gel-forming ability, are very different.



I

Ions play an essential role in the molecular processes associated with conformational transitions and gelation of carrageenans.[1,2,5,7] Recently we suggested that gel formation is a two-step process, consisting of, first, an *intra*molecular transition to an ordered conformation and, second, the formation of ion-selective salt bridges between ordered segments of the chain.[8] This accounts for the ion specificity and the marked difference in the gelling ability of κ- and ι-carrageenans, which both form ordered conformations at low temperature and high ionic strength.[7] Unlike solutions of ι- and λ-carrageenans,[6,9,10] κ-carrageenan gels bind the gel-promoting potassium ions in preference to sodium ions.[8,11] Since Cs^+ ions are similar to K^+ ions in gel-forming ability,[1,2,11] the object here is to obtain information by ^{133}Cs NMR on whether, in harmony with our theory, *inter*molecular site binding of Cs^+ ions takes place in carrageenan gels.

The $^{133}Cs^+$ ion, with nuclear spin $I = {}^7/_2$, has a very small quadrupole moment which results in narrow signals. Nonetheless, its relaxation in the solvated ionic state is entirely quadrupole dominated,[12] and any interaction of the quadrupole moment with the field gradients arising from ionic charges and dipole moments in the polysaccharides will have a dominating influence upon the appearance of the NMR spectrum.[13] In addition, the large Cs^+ ion is easily polarizable, giving comparatively large changes in the chemical shift when its ionic environment or its hydration shell is perturbed by binding to a polyanion. The relatively high sensitivity of ^{133}Cs NMR therefore provides a convenient way of monitoring the binding of counterions to polyanions, through observations on the counterion itself.

Figure 1 shows, on the right, representative NMR spectra of ^{133}Cs obtained from a sample of cesium κ-carrageenate[8] in D_2O at 80 °C (sol state) and, on the left, from the same sample at 25 °C (gel state). These very different spectra are in contrast with those given by the Cs salts of ι- and λ-carrageenans, for which the ^{133}Cs NMR shift remained essentially unchanged by varying the temperature, i.e. close to that at 80 °C in Figure 1. The line shape was also essentially unchanged in spectra of ι- and κ-carrageenans, only a slight broadening due to increased viscosity at low temperature could be observed.

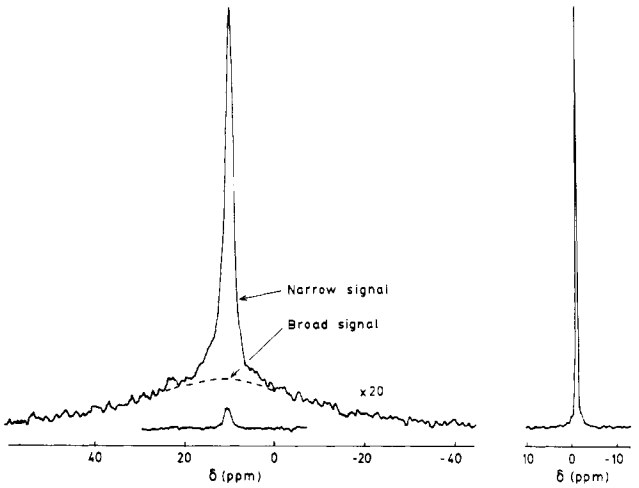


Figure 1. ^{133}Cs NMR spectra of 3% (w/v) cesium κ-carrageenate in D_2O at 80 (right) and 25 °C (left) obtained at 13 MHz with a JEOL FX-100 NMR spectrometer equipped with a multinuclear observation system. Chemical shift is positive downfield relative to $^{133}Cs^+$ ions in 10 mM CsCl in D_2O, contained in a central coaxial tube.

At 80 °C, the ^{133}Cs NMR line from κ-carrageenan (Figure 1, right) is typical of isotropic solutions with rapid molecular motions.[14] A single line constituting 100% of the total intensity is observed. It is shifted slightly upfield (≈1 ppm) relative to a reference line from CsCl in D_2O. Upon lowering the temperature, a very marked and abrupt change in both line shape and line shift (~12 ppm downfield) takes place simultaneously with gelation, indicating changes in the mode of counterion binding (Figure 1, left). While the molecular motion is still isotropic and rapid enough to average the quadrupole interaction to zero, nonexponential relaxation of interacting Cs^+ ions apparently produces two superposed signals: a narrow one accounting for about 20% of the intensity and a broad one accounting for the remainder. This non-Lorentzian line shape is in full agreement with the results of Bull et al.,[15] obtained by numerical solutions of the equations for the quadrupole relaxation of a spin ${}^7/_2$ nucleus engaged in two-site exchange. For long correlation times of the bound ions, $\tau_c \gg 1/2\pi\nu_0$ (ν_0 is the resonance NMR frequency), the transverse relaxation was shown to be a sum of four exponentials with coefficients corresponding to the intensities of the lines found in the quadrupole-split multiplet. The central sharp line in this multiplet, showing 19% of the total intensity,[16] corresponds to the most slowly decaying (i.e., narrow) component which is independent of static quadrupole interaction.[17] The other faster relaxing components give rise to much broader lines in the NMR spectrum. They all have the same resonance frequency as the narrow line and their superposition results in a broad signal on the same position. Thus both the line shape and the intensity predicted by the theory are very close to those observed. Hence, we may conclude that the ^{133}Cs NMR signal from κ-carrageenan in the gel state means that the motion of bound Cs^+ ions is so slow that the condition $\tau_c \gg 1/2\pi\nu_0$ is fulfilled, and the correlation time, τ_c, is greater than 10^{-8} s (ν_0 = 13 MHz). A motional restriction of at least 3 orders of magnitude for bound ions relative to free ions, for which $\tau_c \approx 10^{-11}$ s, is indicative of strong, long-lived binding of the Cs^+ ions at well-defined sites on the carrageenan molecules, where they reside for times longer than 10^{-8} s. It is reasonable to believe that

0024-9297/81/2214-0229$01.00/0 © 1981 American Chemical Society

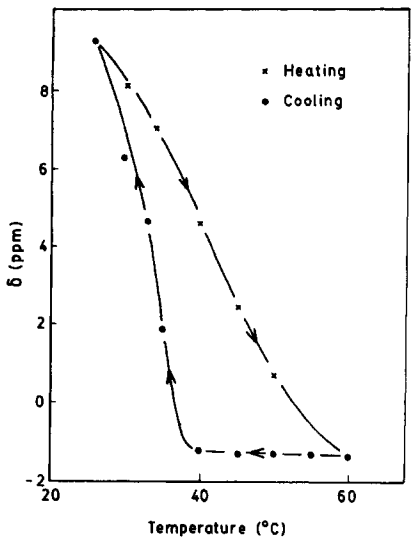


Figure 2. Temperature dependence of ^{133}Cs NMR line shift for the sample defined in Figure 1. The figure shows the effect of cooling and heating through the sol–gel transition, allowing 10 min for equilibration at each temperature. The gel setting temperature is just below 40 °C.

these sites consist of one or more oxygen functions in addition to the sulfate groups arranged in such a way as to fit the size of Cs^+ and K^+ ions.

The residence time is nonetheless short enough to average out the chemical shifts for free and bound ions. The shifts are shown in Figure 2 as a function of temperature for a sample containing 3% (w/v) cesium κ-carrageenate in D_2O. Assuming a two-site model (free and bound, respectively), the downfield shift relative to that at high temperature will be proportional to the fraction of bound ions. Because the affinity for the binding sites seems to be high as shown below (Figure 3), this shift is also proportional to the number of available binding sites. Evaluation of the approximate magnitudes of these quantities would require knowledge of the shift of bound ions, which is unavailable at present. However, since the shift curves (Figure 2) qualitatively closely resemble the behavior of sol–gel transitions, including hysteresis, they demonstrate convincingly that the setting of the gel, i.e., the formation of junctions zones, is accompanied by site binding of counterions.

This idea is further demonstrated in Figure 3, where the ^{133}Cs NMR line shift is plotted against the concentration of cesium κ-carrageenate in D_2O at 25 °C. The sigmoid-shaped curve reflects once more a cooperative process characteristic of a sol–gel transition. The critical concentration for gelation in this system is strikingly demonstrated by the abrupt change of the chemical shift at about 1% (w/v), which is a reasonable value.

Finally, the shifts obtained by addition of LiCl, NaCl, KCl, and CsCl to cesium κ-carrageenate gels (insert in Figure 3) clearly demonstrate selectivity in the binding of the alkali-metal ions. The observed increase of the shift caused by LiCl and NaCl suggests that the number of binding sites for Cs^+ ions increases (by about 20%) when the ionic strength increases, in agreement with gel-strength measurements.[11] Evidently, no significant expulsion of Cs^+ ions from their binding sites is caused by Li^+ or Na^+ ions. On the other hand, addition of KCl or CsCl causes the *fraction* of bound Cs^+ ions to decrease as judged by the observed decrease of shifts (Figure 3). As the reduction induced by these two ions is almost identical, they must compete for binding sites having very similar binding capabilities. Quantitatively, the population-weighed, average

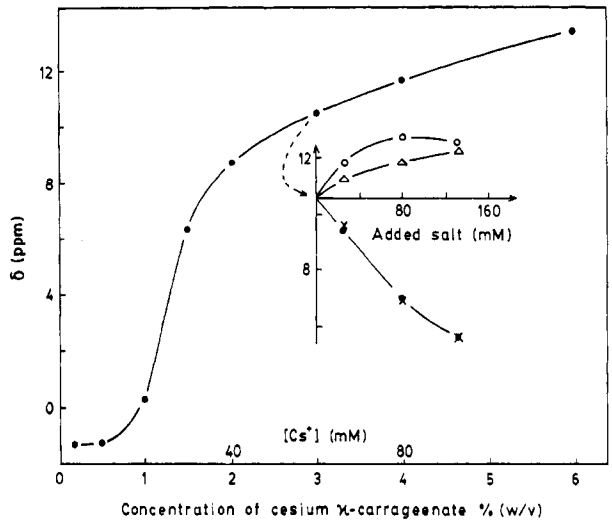


Figure 3. ^{133}Cs NMR line shift as a function of concentration of cesium κ-carrageenate in salt-free D_2O solution at 25 °C. Equivalent Cs^+ concentration is indicated on the abcissa axis. The insert shows how the shift for a 3% (w/v) cesium κ-carrageenate gel is affected by addition of salts: (○) NaCl; (△) LiCl; (×) KCl; (●) CsCl.

^{133}Cs NMR shift drops monotonically by about 30% when the Cs/carrageenan ratio is increased to twice its equivalent value, which is somewhat less than the 50% expected if the number of bound ions stays constant. However, the estimated increase in the fraction of bound ions, about 40% provided the binding sites remain unchanged, may be mainly accounted for by taking into account that Cs^+ ions are much more efficient that Na^+ ions in their gel-promoting effect and consequently produce more junction zones and probably more binding sites. It therefore seems that the sites are almost fully occupied by Cs^+ ions, which means that the affinity must be high.

The main conclusions are as follows: The gelation of κ-carrageenan produces highly selective binding sites for alkali metal ions, in which K^+ and Cs^+ ions bind much more strongly than Li^+ or Na^+. The correlation between the ^{133}Cs NMR line shift and the introduction of intermolecular contact zones in gel state supports our idea[8] that ion-selective salt bridges constitute the junctions in κ-carrageenan gels.

References and Notes

(1) Smith, D. B.; Cook, W. H. *Arch. Biochem. Biophys.* **1953**, *45*, 232.
(2) Smith, D. B.; Cook, W. H.; Neal, J. L. *Arch. Biochem. Biophys.* **1954**, *53*, 192.
(3) Anderson, N. S.; Rees, D. A. *J. Chem. Soc.* **1965**, 5880.
(4) Anderson, N. S.; Doland, T. C. S.; Rees, D. A. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2173.
(5) Reid, D. S. In "Ions in Macromolecular and Biological Systems" (Proceedings of the 29th Symposium of the Colston Research Society); Evrett, D. H., Vincent, B., Eds.; Scientechnica: Bristol, 1978.
(6) Rinaudo, M.; Karimian, A.; Milas, M. *Biopolymers* **1979**, *18*, 1673.
(7) Morris, E. R.; Rees, D. A.; Norton, I. T.; Goodall, D. M. *Carbohydr. Res.* **1980**, *80*, 317.
(8) Smidsrød, O.; Andresen, I.-L.; Grasdalen, H.; Larsen, B.; Painter, T. *Carbohydr. Res.* **1980**, *80*, C11.
(9) Pass, G.; Phillips, G. O.; Wedlock, D. J. *Macromolecules* **1977**, *10*, 1977.
(10) Kowblansky, M.; Tomasula, M.; Ander, P. *J. Phys. Chem.* **1978**, *82*, 1491.
(11) Smidsrød, O. "IUPAC 27th International Congress of Pure and Applied Chemistry"; Varmavuori, A., Ed.; Pergamon Press: Oxford and New York, 1980; p 315.
(12) Wehrli, F. W. *J. Magn. Reson.* **1978**, *30*, 193.
(13) Forsén, S.; Lindman, B. *Chem. Br.* **1978**, *14*, 29.

(14) Lindman, B.; Forsén, S. "Chlorine, Bromine and Iodine NMR, Physicochemical and Biological Applications"; Springer-Verlag: Heidelberg, 1976.
(15) Bull, T. E.; Forsén, S.; Turner, D. L. *J. Chem. Phys.* **1979**, *70*, 3106.
(16) Pake, G. E. *Solid State Phys.* **1956**, *2*, 1.
(17) Abragam, A. "The Principles of Nuclear Magnetism"; Clarendon Press: Oxford, 1961.

Hans Grasdalen* and Olav Smidsrød

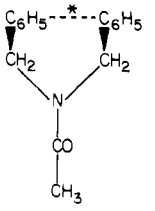
Institute of Marine Biochemistry
The University of Trondheim
N-7034 Trondheim-NTH, Norway

Received June 20, 1980

CORRECTIONS

T.-P. Liao and H. Morawetz*: On the Nonexistence of Crankshaft-like Motions in Dilute Solutions of Flexible-Chain Molecules. Volume 13, Number 5, September–October 1980, page 1228.

The formula of the excimer in the right column of p 1229 should be



Ettore Benedetti,* Anna Ciajolo, Benedetto Di Blasio, Vincenzo Pavone, Carlo Pedone, Claudio Toniolo, and Gian Maria Bonora: Linear Oligopeptides. 59.[1] Stereochemical Analysis of *N-tert*-Butyloxycarbonyl-L-prolylsarcosine and *N-tert*-Butyloxycarbonylsarcosylsarcosine in the Solid State and in Solution. Volume 12, Number 3, May–June 1979, page 438.

Due to computational errors the atomic coordinates of C_1', H(1)–C_1^{α}, and H(2)–C_1^{α} of *t*-Boc-Sar-Sar-OH given in Table II are incorrect.

The correct coordinates are the following:

	x	*y*	*z*
C_1'	0.2496	0.1471	−0.1751
H(1)-C_1^{α}	0.4338	0.1603	−0.1705
H(2)-C_1^{α}	0.3975	0.0121	−0.1146