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Effects of added galacto- and gluco-mannans on the NMR spectra of ^{133}Cs ions in kappa-carrageenan gels

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Abstract

NMR chemical shifts and line-widths of ^{133}Cs ions were measured in kappa-carrageenan gels containing galacto- or gluco-mannans, and compared with measurements on kappa-carrageenan alone. In the mixed gels, as in pure kappa-carrageenan, large changes in the NMR parameters were found to accompany the onset of helix formation for kappa-carrageenan, as obtained independently from polarimetry or differential scanning calorimetry. The shift changes on helix formation in the mixed gels were very similar to those in kappa-carrageenan alone, indicating that the ordered conformation of kappa-carrageenan, and its degree of specific ion binding, was the same in all systems. A much enhanced line-broadening was seen in gels containing carob galactomannan or konjac glucomannan, which are known to form synergistic gels in the mixtures with kappa-carrageenan, while other additives (guar galactomannan or agarose) had a small or insignificant effect. The observations are interpreted in terms of an association between mannans and (aggregated) kappa-carrageenan helices in the synergistic gel mixtures.

Keywords: Polysaccharide interactions; Kappa-carrageenan; Galactomannan; Glucomannan; ^{133}Cs NMR

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1. Introduction

The gelling properties of certain polysaccharides can be improved by the addition of certain galacto- or gluco-mannans [1–3]. The mixed gels become less brittle and more elastic, and it is possible to obtain an enhanced elastic modulus, at a fixed total concentration in the mixture. Thus, expensive polysaccharides can be partially replaced by cheaper ones, while the performance is kept or even improved. Consequently, these kinds of mixtures are attractive in many applications. The most familiar and widely exploited mixed polysaccharide gels are those involving carob galactomannan (locust bean gum), or konjac glucomannan, in combination with xanthan or with certain red seaweed galactans (kappa-carrageenan, furcellaran or agarose). Galactomannans consist of linear chains of β-(1 → 4)-linked D-mannopyranosyl residues, to which D-galactopyranosyl groups are α-(1 → 6)-linked as single-unit side chains. The D-galactose distribution has been shown to be irregular with a high proportion of substituted doublets, a smaller amount of triplets, and an absence of longer blocks of substitution [4]. Konjac glucomannan has a more complex structure [5]. Its main chain consists of β-(1 → 4)-linked D-glucose and D-mannose, where the D-mannose-to-D-glucose ratio is 1.6:1 and the residues are randomly distributed. Branching occurs through C-3 of both mannose and glucose residues approximately every ten sugar residues and the branch may be up to 11–16 sugar units in length. Its precise composition is not known. Approximately 1 in 19 sugar units is acetylated.

Although the synergistic effect of the galacto- and gluco-mannans in mixed gels is well known, the gel structure is still a matter of debate. Cairns et al. [6] discussed four possible structures for mixed polysaccharide gels. In three of the structures, i.e., interpenetrating networks, phase-separated networks and coupled networks, both polysaccharides contribute to the network(s), whereas the fourth structure involves a single polysaccharide network which merely contains the second polysaccharide dissolved in the intervening solvent. All of these structures have been considered for the mixtures involving galacto- and gluco-mannans. Indeed, it has been suggested [7] that the mode of interaction may be different for mixtures with xanthan than for mixtures with the red seaweed galactans. In the present work, we focus on the interactions of galacto- and gluco-mannans with kappa-carrageenan. Kappa-carrageenan is characterized by an alternating disaccharide unit of β-(1 → 3)-linked D-galactose-4-sulphate and α-(1 → 4)-linked 3,6-anhydro-D-galactose. This polysaccharide undergoes a coil–helix conformational transition, followed by gelation, promoted by lowering the temperature in the presence of ions such as potassium or cesium.

Earlier studies by our laboratories examined kappa-carrageenan/galactomannan mixtures by means of ^{13}C NMR [8], rheological measurements [9], small angle X-ray scattering [10], and molecular modeling [11,12], while kappa-carrageenan/glucomannan mixtures were investigated by electron spin resonance spectroscopy (ESR), differential scanning calorimetry (DSC), and rheological methods [13,14]. The ^{13}C NMR data showed that the unsubstituted (''smooth'') zones of galactomannans lost their mobility when the mixtures were cooled below the transition temperature of kappa-carrageenan, and similar results were found by ESR for the case of konjac glucomannan. These findings strongly indicate that the mannan chains take part in some association process as the mixed gel is formed. The measurements could not, however, distinguish between a self-association of the mannan chains or the formation of mixed aggregates, since the possible effects of the mannan chains on the state of the kappa-carrageenan chains was not probed. In the present work, we use ion NMR to provide such information for the polysaccharide samples previously studied by other methods. Ion NMR has proven to be particularly useful to probe structure and interactions in kappa-carrageenan gels [15–22]. For the gel-promoting cations, changes in the chemical shift and large enhancements of the line-widths occur on conformational ordering, indicating a specific binding of these ions to the helical conformation of kappa-carrageenan. It is important to note that these NMR effects are only seen for the gel-promoting cations, and only for samples containing helices of the lower charged carrageenans. Neither random coil molecules nor pure iota-carrageenan in any conformational state [21] give rise to such effects. This indicates that the ion binding is quite structure-specific, so that counterion NMR may be used as a sensitive probe for the carrageenan structure. Moreover, the shift and line-width data give complementary information. If fast exchange conditions prevail, the observed shift is simply the population-weighted average of the intrinsic chemical shifts of the free and bound ions, irrespectively of the dynamics of these ions. The line-widths, on the other hand, provide additional information on the dynamics, presumably the exchange rate, of the bound ions. In the present study, the ^{133}Cs ion was preferred over the other possible candidates (^{87}Rb and ^{39}K) since its small quadrupole moment gives rise to comparatively narrow lines and, consequently, a possibility to accurately determine small shift changes.

2. Experimental

Materials.—Kappa-carrageenan (from *Eucheuma cottonii*), agarose (from *Gelidium sesquipedale*, carob (from *Ceratonia siliqua*), and guar (from *Cyamopsis tetragonolobus*) galactomannans were provided by Sanofi Bio Industries (France). All were used after purification according to methods already described [23,24]. The average molecular masses of the samples were $M_v = 510\,000$ for kappa-carrageenan (sodium form, from Mark–Houwink parameters [25] in 0.1 M NaCl at 25°C, with $[\eta] = 8.20$ dL/g), $M_v = 112\,000$ for agarose (from Mark–Houwink parameters [26] in 0.75 M NaSCN at 35°C, with $[\eta] = 3.00$ dL/g), $M_v = 1\,960\,000$ for guar galactomannan (from the Robinson equation [27] in 0.1 M NaCl at 25°C, with $[\eta] = 13.48$ dL/g), and $M_v = 880\,000$ for carob galactomannan (from the modified Robinson equation [28] with $[\eta] = 9.06$ dL/g). The galactose–mannose ratios were 38:62 and 23:77 for guar and carob galactomannans, respectively. The cesium salt of kappa-carrageenan was obtained by exact neutralization with CsOH of the acidic form, after percolation through a column of ion-exchange IR 120 (H^+) resin. The solution was concentrated using a rotary evaporator and then freeze-dried. A purified form of konjac mannan was obtained from FMC Marine Colloids Division. The average molecular mass was found to be 450 000 by multi-angle laser light scattering using the Malvern PCS 100SM. In the mixtures with konjac mannan, a kappa-carrageenan sample from FMC Marine Colloids Division was used. This sample was converted to the sodium salt form by ion exchange using Amberlite IR 120 ion-exchange resin. The average molecular mass was found to be 330 000 by multi-angle laser light scattering using a Wyatt Dawn model laser photometer. Aqueous solutions of the polysaccharides were prepared by adding solvent (water or salt solution) to the dry polysaccharide(s) followed by heating at 95°C. The hot

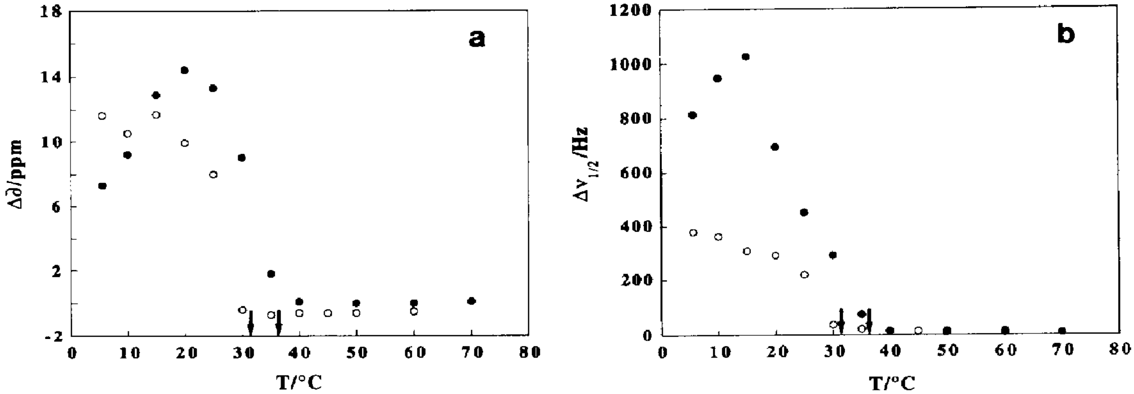

Fig. 1. Temperature dependence (on cooling) of the relative chemical shift (a) and line-width (b) of $^{133}Cs^{+}$ for 1% Cs–kappa-carrageenan alone (○) and mixed with 2% carob galactomannan (●). Arrows denote onset of conformational order, as obtained by optical rotation.

solutions were poured into standard 12-mm NMR tubes. The sample tubes were flame-sealed and subsequently heated at ca. 100°C, to ensure that all of the sample collected at the bottom of the tube. All polysaccharide concentrations are given as mass per cent (%) of the total sample mass.

Methods.—^{133}Cs NMR spectra were recorded at 47.45 MHz for samples in 12-mm tubes with a Nicolet NIC-360 spectrometer, using 4K data points and a 10 kHz spectral width (1 kHz for the reference solutions of simple salts). The observed relative chemical shifts of ^{133}Cs in kappa-carrageenan samples, $\Delta\delta_{obs}$, were obtained as the difference $\delta_{obs} - \delta_{ref}$, where δ_{obs} and δ_{ref} are the shifts, measured at the same temperature, in the sample and in a reference solution containing the same amount of added salt. Line-widths at half height, $\Delta\nu_{1/2}$, were obtained from Lorentzian fits to the absorption spectra. For the gelled samples, the contribution from magnetic field inhomogeneity to the line-widths was negligible. DSC measurements were carried out using a Setaram micro DSC calorimeter fitted with 1-mL sample and reference cells. Cooling and heating curves for carrageenan and carrageenan/konjac mannan mixtures in the presence on 20 mM CsCl were obtained between 10 and 85°C at a scan rate of 0.1°C per minute. The samples were initially subjected to a prior heating and cooling cycle to ensure the same thermal history. The optical rotation was determined at 435 nm for a 5-cm pathlength.

3. Results and discussion

Effects of conformational transition on ^{133}Cs NMR parameters.—Grasdalen and Smidsrød [15] have shown that the thermally induced conformational transition of kappa-carrageenan is reflected in the chemical shift and line-width of the cesium ion. Figs. 1 and 2 show that these effects occur also in mixtures with carob galactomannan or konjac glucomannan. Just as in pure kappa-carrageenan gels, the ^{133}Cs line-width increases rapidly and the lineshape becomes visibly bi-Lorentzian [15] as the temperature is decreased below T_o, the temperature of onset of the conformational transition. Although, as has been shown previously [13,14,29], the transition is shifted to higher temperatures in the mixtures, the

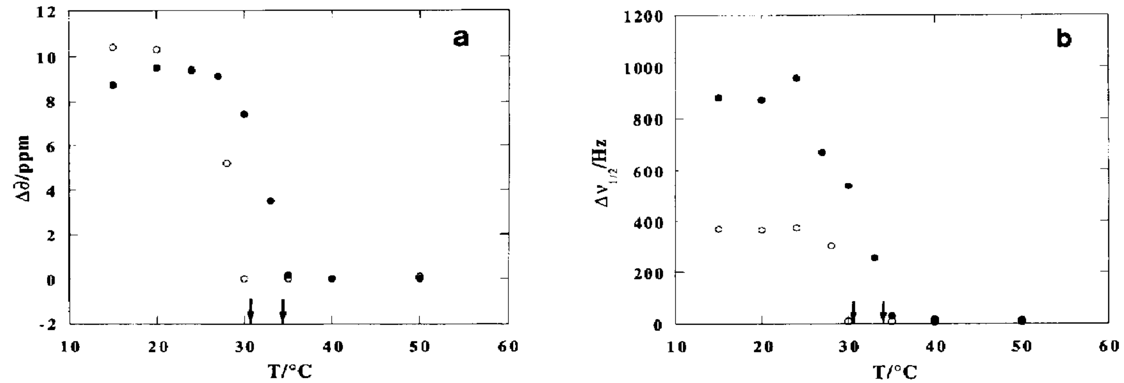

Fig. 2. Temperature dependence (on cooling) of the relative chemical shift (a) and line-width (b) of $^{133}Cs^+$ of 0.76% Na–kappa-carrageenan in 20 mM CsCl alone (○) and mixed with 0.76% konjac glucomannan (●). Arrows denote onset of conformational order, as obtained from the DSC data in Fig. 3.

good correlation remains between T_o (as obtained from optical rotation or DSC) and the onset of significant changes of the NMR parameters (Figs. 1–3). For the mixture with konjac mannan, the entire transition on cooling was monitored by DSC (Fig. 3). It has been suggested [13] that, since the transition in the mixed system occurs at a higher temperature than the transition of kappa-carrageenan alone, it might involve some structure of kappa-carrageenan other than the ordinary helix. Our NMR results show, however, that the transition, which is seen in DSC and optical rotation, results in the formation of a structure which has the same ion-binding properties as the normal kappa-carrageenan helix. As the ion binding is quite structure specific, this is a strong indication that the ordered conformation formed in the mixture is, indeed, the normal kappa-carrageenan helix. Moreover, the magnitude of the shifts observed in the mixtures is very nearly the same as in the corresponding systems containing kappa-carrageenan alone, meaning that the fraction of bound ions is virtually the same in the two types of systems. This argues against the existence of types of ordered or complexed kappa-carrageenan, other than the normal helix, in the mixtures.

On the other hand, the presence of carob galactomannan or konjac glucomannan chains clearly gives rise to an increase in the $^{133}Cs^+$ line-width at temperatures where kappa-carrageenan chains are partially or totally in the ordered form (Figs. 1b and 2b). Since the fraction of bound ions is unaffected (cf. the shift data), the line-broadening must mean that

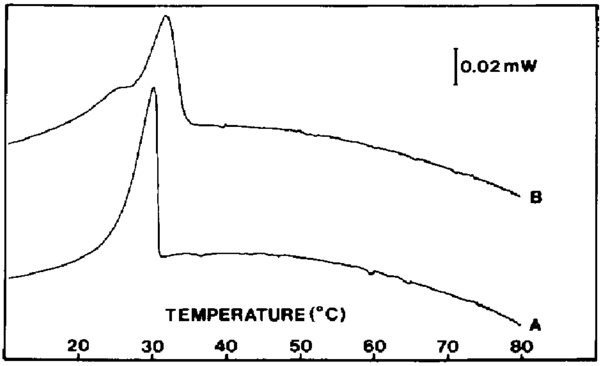

Fig. 3. DSC cooling curves for 0.76% Na–kappa-carrageenan in 20 mM CsCl, alone (A) and mixed with 0.76% konjac glucomannan (B).

the presence of the mannan chains gives rise to a change in the dynamics of the bound ions. A likely possibility is that the effective exchange of the ions between the bound and the free sites is being slowed down in the mixed gels [21]. This interpretation is supported by the fact that, at low temperatures, both the shifts and the relaxation rates pass through a maximum with decreasing temperature in the mixed gels, while a monotonic increase is seen for pure kappa-carrageenan. The maximum indicates that, at sufficiently low temperatures in the mixtures, the exchange of the bound cesium ions is no longer rapid on the NMR timescale. A similar shift maximum was seen previously for aggregated samples of furcellaran, and was given a similar interpretation [22]. Regardless of the detailed interpretation of the line-broadening (cf. the complex variations of line-width with ion dynamics predicted in the theoretical works by Westlund and co-workers [30–32]), it seems difficult to escape the conclusion that the changes in the dynamics of the bound ions must be caused by changes in the super-molecular organisation of the kappa-carrageenan helices in the mixed gels. The previous NMR and ESR results have shown that the helix formation of kappa-carrageenan in mixed gels is accompanied by an association of the mannan chains. Our ^{133}Cs NMR results now indicate that also the mode of association of the kappa-carrageenan helices changes in the mixture. The simplest interpretation of all these data is that they signify the formation of mixed aggregates in the mixed gels.

A low-temperature shoulder is seen in the DSC curve of the konjac mannan/kappa-carrageenan mixture (Fig. 3). This corresponds to the low-temperature transition reported earlier for similar mixtures in the presence of potassium ions, and attributed to gelation/aggregation of excess carrageenan [13,14]. Unfortunately, this transition is not as well resolved for the cesium form. This may also be the reason why no indication of a two-step transition is found in the NMR data of the sample (Fig. 2).

Effects of amount and type of added polysaccharide.—To obtain a more detailed picture of the interaction between kappa-carrageenan and the synergistic additives, we studied mixtures where the types and amounts of added polysaccharide were varied. These studies were all performed at 15°C, where, according to the shift data of Figs. 1a and 2a, the conformational transition is essentially complete. Fig. 4 compares the effects of added guar and carob galactomannan on the Cs NMR parameters. The purpose of this experiment was to compare galactomannans having small and large effects, respectively, on the mixed gel

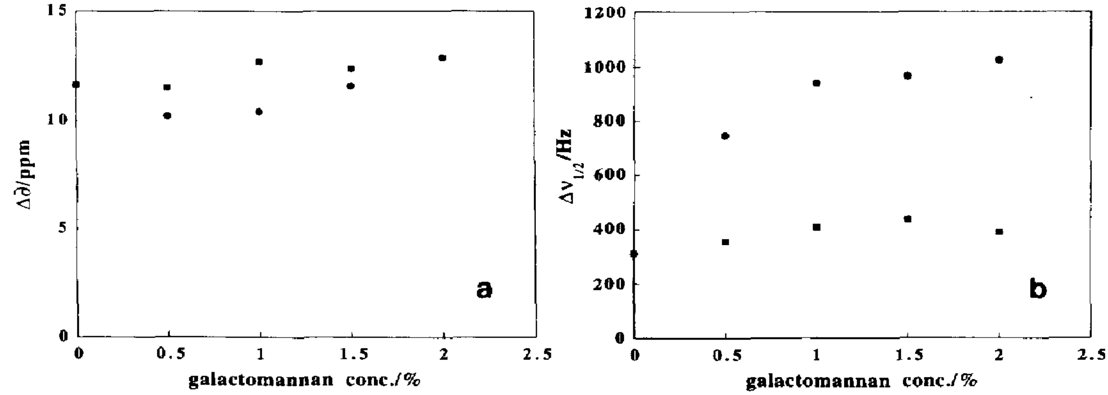

Fig. 4. Chemical shifts (a) and line-widths (b) at 15°C of $^{133}Cs^+$ in mixed gels of Cs–kappa-carrageenan (1%) with added carob (●) or guar (■) galactomannans.

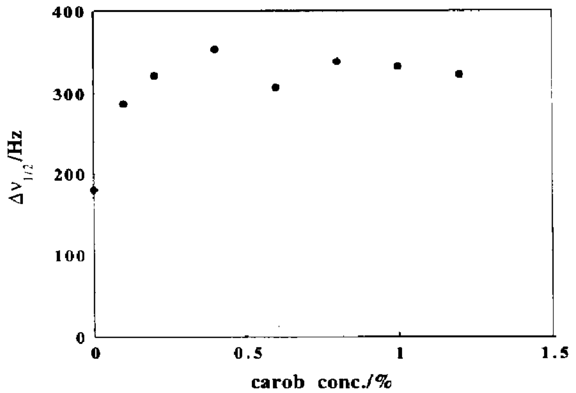

Fig. 5. Line-widths at 15°C of $^{133}Cs^+$ in mixed gels of Na–kappa-carrageenan (0.2%) in 20 mM CsCl with added carob galactomannan.

properties. Carob galactomannan has a high proportion of smooth zones and a large synergy with kappa-carrageenan, whereas guar galactomannan, with fewer smooth zones, displays much less synergy. The shift results (Fig. 4a) show, as expected (cf. the previous section), that neither of the galactomannans has a significant influence on the fraction of bound cesium ions. The line-widths, on the other hand, show pronounced differences for the two galactomannans (Fig. 4b). The large line-broadening seen with added carob is replaced by a much weaker effect for guar, meaning that whatever the molecular interaction is that causes the line-broadening, it is substantially weaker with guar gum. For added carob, the line-width seems to level off at high galactomannan concentrations. This is consistent with a progressive ''saturation'' of the carrageenan helices at high galactomannan/carrageenan ratios. We found it very difficult to prepare homogeneous samples with still higher concentrations of galactomannans. Therefore, samples with higher galactomannan/carrageenan ratios were made by lowering (to 0.2%) the concentration of kappa-carrageenan. To these samples, 0.02 M CsCl was added, to ensure complete conversion to the helical form. Fig. 5 shows that these measurements confirm the levelling off of the line-broadening at high kappa-carrageenan/galactomannan ratios.

It could be argued that the enhancement of the cesium line-width in the mixtures with carob could be a nonspecific effect of a change in gel structure, [33] due to the formation of a secondary network of the carob chains. The lack of a significant effect with added guar would then simply be due to a lack of self-association of the guar chains. A few experiments were performed in order to test this alternative hypothesis. Since agarose is known to give rise to an interpenetrating network when mixed with kappa-carrageenan, [34] we measured the cesium ion NMR in a few mixed gels with agarose in 0.02 M CsCl. The results are

Table 1
^{133}Cs NMR line-widths (Hz) at 15°C for various polysaccharide solutions and gels in 20 mM CsCl

System	1% agarose	2% carob	1% agarose + 1% carob	1% kappa-carrageenan	1% kappa-carrageenan + 1% agarose	1% kappa-carrageenan + 2% agarose
Line-width	< 10	< 10	< 10	410	550	540

Table 2
^{23}Na NMR line-widths (Hz) at 15°C for Cs kappa-carrageenan gels in 5 mM NaCl

System	1% kappa-carrageenan	1% kappa-carrageenan + 1% carob
Line-width	32	45

shown, together with results for relevant reference systems, in Table 1. It is seen that agarose only produces a small line-broadening, comparable to the effect of guar, despite the fact that the agarose network is heavily aggregated. We also measured the ^{23}Na ion line-width in gel samples containing a small amount of added NaCl (Table 2). These measurements provide a different test for possible nonspecific effects of a secondary network, since the sodium ion does not bind specifically to kappa-carrageenan [20]. Table 2 shows that only a small line-broadening of the sodium ion is found in a gel containing 1% carob galactomannan. This confirms that the slow dynamics, which give rise to a large line-broadening in the mixed gels, only involve site-bound ions. The results of these simple experiments clearly speak against an interpretation of the effect of carob as an effect of a secondary interpenetrating network.

A last possibility to be considered is that all the ^{133}Cs NMR effects in the synergistic gels could be due exclusively to changes in the self-association of the carrageenan chains, induced by an effectively repulsive, rather than an attractive, interaction with the synergistic additives. Obviously, this possibility cannot be excluded from the NMR data alone. It is quite difficult to understand, however, why a strong repulsion should occur precisely with carob galactomannan and konjac glucomannan, while no or weak effects are seen for the chemically similar guar galactomannan (which has a higher molecular weight and, therefore, would be expected to give a stronger segregation) or for a highly self-aggregating additive like agarose. Adding available experimental evidence, we find that the onset of an increase in G' and G'' in the synergistic kappa-carrageenan/mannan gels coincides with the onset of the transition seen with DSC [13], which, in turn, coincides with changes in optical rotation. NMR [8] and ESR [13,14] data show, furthermore, that these events are also correlated with a partial immobilization of mannan chains, and with changes in the mode of association of the kappa-carrageenan chains (this work). The coincidence of association phenomena involving both types of chains is difficult to reconcile with models assuming a repulsion between unlike chains, while it is quite natural for a process where mixed aggregates are formed.

X-ray diffraction studies from mixed carrageenan/mannan gels only give the patterns characteristic of the carrageenan component, and thus give no evidence of the formation of mixed aggregates [6]. On the other hand, both NMR and ESR undisputably show that the mannan chains do participate in some association, leading to a reduced mobility of the chains. Evidently, this mode of association is not seen in the X-ray patterns. Ascribing the spin resonance evidence to mannan self-association does not seem to solve the problem; it is not obvious why this mode of association would be more invisible in X-ray diffraction than would the formation of mixed aggregates. A detailed discussion of the possible nature of the mixed aggregates in kappa-carrageenan/mannan gels has recently been presented by two of the present authors [14]. There, it was suggested that the mixed gels contain bundles of self-aggregated helices covered with surface-adsorbed mannan chains, and, moreover, that the adsorption of the mannan chains also influenced the self-association of the kappa-carrageenan helices. Provided that the mixed aggregates involve *disordered* mannan chains [6,35] (to account for their invisibility in X-ray diffraction patterns), this model seems quite consistent with available data, including the data presented in the present article. In particular, a slowing down of the exchange rate of cesium ions bound to the kappa-carrageenan helices (cf. the line-broadening and the nonmonotonic temperature dependences of the chemical shifts and line-widths of $^{133}Cs^+$ spectra in the mixed gels) seems a likely consequence of the formation of such mixed aggregates.

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