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Relation Between the Molecular Structure and Mechanical Properties of Carrageenan Gels*

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ABSTRACT

Carrageenan extracted from Eucheuma cottonii *was analyzed by enzymic hydrolysis, KCl fractionation,* ^{13}C *and* ^{1}H *NMR spectroscopy.* ^{13}C *and* ^{1}H *NMR of the enzymic resistant fraction and of the KCl-soluble fraction prove that the carrageenan extracted from* Eucheuma cottonii *is composed of several polysaccharides. The major component is kappa-carrageenan and the minor ones are irregular galactans partially methylated, containing a large proportion of the iota-carrageenan form.*

INTRODUCTION

Carrageenans are sulfated galactans extracted from many species of red algae, the Rhodophyceae (Rees, 1962; Kloareg, 1981). They are composed of D-galactose residues linked alternately with α-1,3 and β-1,4 linkages. These sulfated galactans are classified according to the presence of the 3,6-anhydrogalactose on the 4-linked residue and the position and the number of sulfate groups (Rees, 1969; McCandless & Craigie, 1979). The more important types of carrageenans have been determined as the kappa-, iota-, lambda-, mu- and nu-carrageenans. Mu- and nu-carrageenans are biological precursors of kappa-carrageenans (Lawson & Rees, 1970; Wong & Craigie, 1978). Kappa- and iota-carrageenans contain the 3,6-anhydro unit (Fig. 1) and are gelling polymers, but lambda-carrageenan with only galactose groups is a thick-

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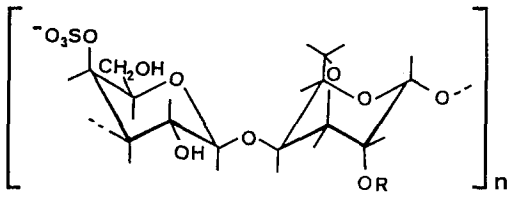

Fig. 1. Primary structure of kappa (R = H)- and iota (R = SO_3^-)-carrageenans.

ening polymer. Generally, carrageenans extracted from algae do not have an ideal structure and are described as hybrid polymers (Bellion *et al.*, 1982; Greer *et al.*, 1984). For example, carrageenans from species of *Eucheuma* contain in variable percentages kappa-, iota-, mu- and nu-carrageenans but the composition varies with the species (Di Ninno & McCandless, 1978; Bellion *et al.*, 1982).

The term hybrid is not precise enough to describe the different possible structures which can occur in the polysaccharides extracted from an alga. If an hybrid structure exists, for example kappa-iota with kappa as the major component, three different combinations can explain this result (Fig. 2). We can imagine many short blocks of iota structure dispersed in the chain of kappa or some long sequences of iota in the chain of kappa or a mixture of kappa and iota chains.

Mechanical properties of gels obtained from carrageenans depend on the type of carrageenan and its composition. So, in this study, we have tried to determine the molecular structure of carrageenan from *Eucheuma cottonii* and to relate the molecular structure of this car-

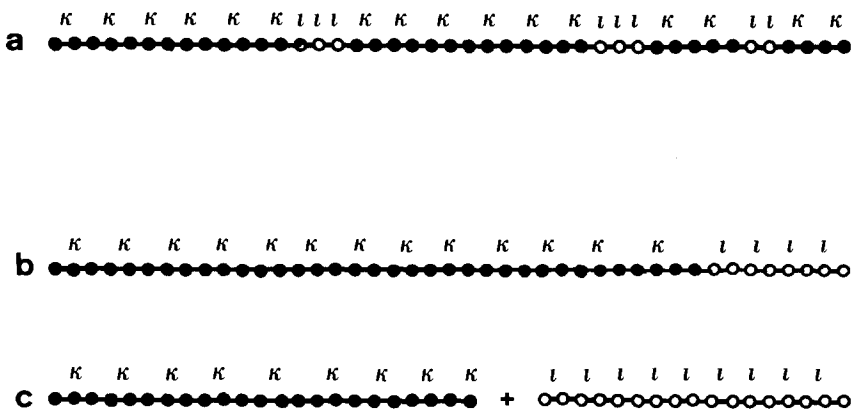

Fig. 2. Possible structures to explain the heterogeneity of the extract of *Eucheuma cottonii*. (a) Hybrid structure — random short sequences of iota in a chain of kappa; (b) hybrid structure — long sequence of iota in a chain of kappa; (c) mixture of pure chains of kappa and pure chains of iota.

rageenan to the mechanical properties of the gels obtained. This carrageenan was identified as a polysaccharide with kappa-carrageenan as the major component and iota-carrageenan as the minor component. For this purpose, enzymic hydrolysis and KCl fractionation were used to determine the structure of the extract from *E. cottonii* and mechanical properties were obtained by compression tests.

EXPERIMENTAL

The alkali-treated extracts from *E. cottonii* (EC), *E. spinosum* (ES), the alkali-treated extract from *Chondrus crispus*, the extract from *C. crispus* and the alkali-treated extract from *Gigartina stellata* were supplied from MRS (Baupte, France).

The weight average molecular weights of the samples were determined by size exclusion chromatography on two columns (Shodex B 804 and B 805; 50 cm length) coupled to a Jobin Yvon Iota differential refractometer and a Chromatix KMX-6 low angle light scattering photometer according to Lecacheux *et al.* (1985).

Kappa-carrageenase was prepared from the cell-free medium of *Pseudomonas carrageenovora* grown in kappa-carrageenan from EC according to McLean & Williamson (1979). The enzymic resistance fraction was separated from salt and oligomers by ultrafiltration (Amicon XM 300 membrane). The fractionation of EC was carried out in the following way: on a solution of EC (5 g litre^{-1}), a KCl solution was added at room temperature to obtain a salt concentration and a final polymer concentration of 0·03 M and 2 g litre^{-1} respectively. After one night at room temperature the mixture was centrifuged for 1 h at 30 000 *g*. The gel phase and the supernatant after separation were dialyzed and freeze-dried. ^{1}H NMR spectra of the two fractions from KCl fractionation were recorded on a Bruker WH 400 spectrometer. The other ^{1}H NMR spectra and the ^{13}C NMR spectra were recorded on a Bruker AM 300 spectrometer. The spectra were recorded in D_2O solutions at 80°C. The concentrations were 5 g litre^{-1} for ^{1}H NMR spectra and 40 g litre^{-1} for ^{13}C NMR spectra. The optical rotation was measured at 365 nm on a Perkin–Elmer 241 polarimeter with a 100 mm thermostated cell.

The samples of gel were prepared as follows: the hot solution of carrageenan was poured into a glass tube of diameter 17 mm. After cooling at room temperature the gel was cut into small cylinders (diameter = 17 mm and height = 17 mm) and the samples were relaxed in the solvent overnight for kappa-carrageenan and one week for iota-carrageenan or the blend of kappa- and iota-carrageenan. Young's

modulus E and the yield stress F_m were obtained at room temperature by mechanical tests using a 4301 Instron machine as previously described (Rochas & Landry, 1988). The intrinsic viscosities were determined at 25°C in 0·05 M LiCl for the iota-carrageenan fraction and in 0·1 M NaCl for the kappa-carrageenan fraction. Gas chromatography on the alditol acetate sugar was performed after total acidic hydrolysis as described by Sawardeker *et al.* (1965). Films for infrared analysis were obtained as described previously (Rochas *et al.*, 1986).

RESULTS

Molecular structure of carrageenan from *Eucheuma cottonii*

The infrared spectra of EC (Fig. 3) show a band of low absorbance at 805 cm^{-1}. This band is due to the 3,6-anhydrogalactose-2-sulfate unit (Anderson *et al.*, 1968) and consequently some iota-carrageenan units occur in EC. This is supported by the chemical shifts (Rochas *et al.*, 1983; Greer *et al.*, 1985) of the signals of the ^{13}C NMR spectra (Fig. 4) confirmed by the chemical shifts (Welti, 1977) of the signals of the ^{1}H spectrum (Fig. 5). In addition the presence of methyl groups is shown as

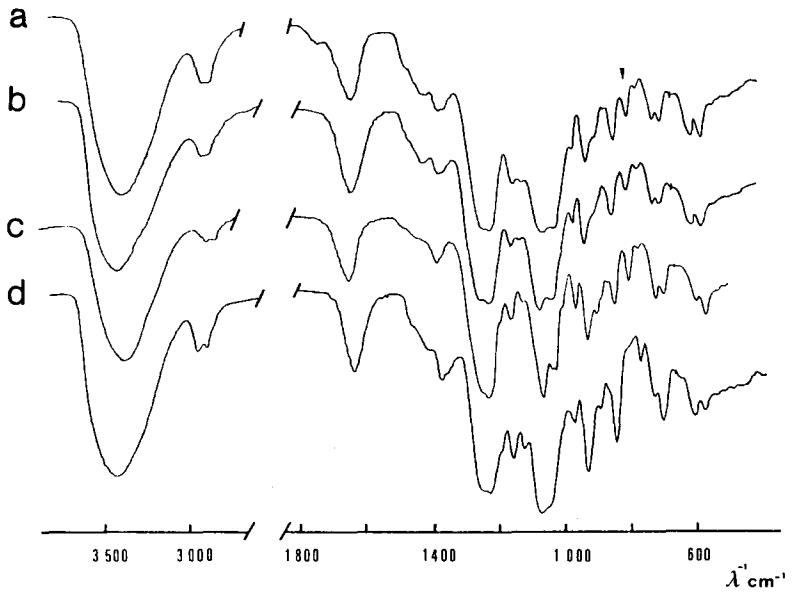

Fig. 3. Infrared spectra. (a) KCl-soluble fraction; (b) enzymic resistant fraction; (c) iota-carrageenan (*Eucheuma spinosum*); and (d) kappa-carrageenan (*Eucheuma cottonii*).

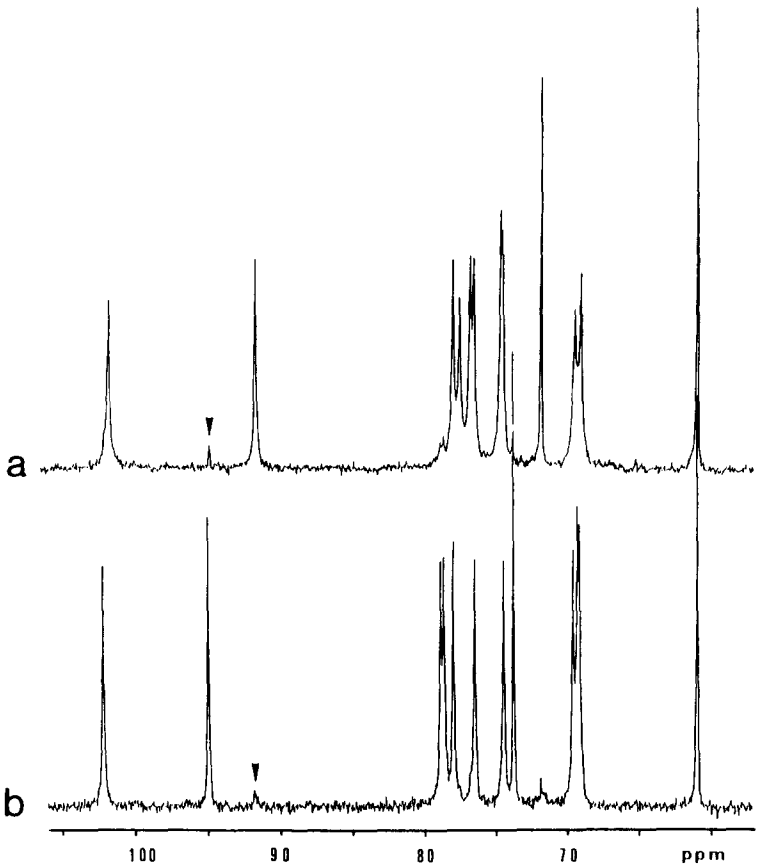

Fig. 4. ^{13}C NMR spectra of polysaccharides from *Euchema spinosum* (a) and *Eucheuma cottonii* (b).

previously reported by Bellion *et al.* (1983) for the biological precursor of *E. cottonii*. From the different spectra of EC recorded, the molar fraction of methylated units is approximately 0·03 and that of iota-carrageenan units is 0·1. but we do not know if this iota-carrageenan is included or not in the hybrid structures represented in Figs 2(a) and 1.

Enzymic hydrolysis gives an initial answer to this question. After this hydrolysis the extract of EC is separated into oligomers and an enzymic resistance fraction (ERF). The ERF fraction represents 10·8% (w/w) of EC. The intrinsic viscosity and the weight average molecular weight of ERF are 280 ml g^{-1} and 220 000 respectively. These values are quite a large proportion of the intrinsic viscosity and the weight average molecular weight of the extract of EC, 819 ml g^{-1} and 690 000 respectively. Infrared spectra (Fig. 3) and ^{1}H spectra (Fig. 5) show that ERF contains a significant proportion of iota-carrageenan units and a high

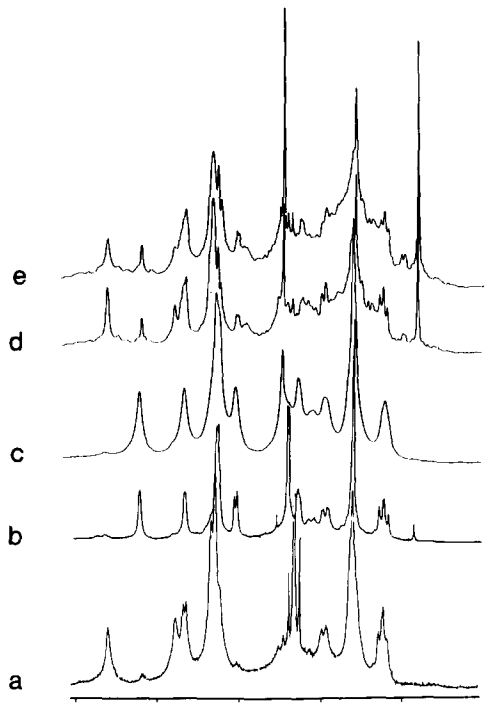

Fig. 5. ^{1}H NMR spectra of polysaccharides from *Eucheuma spinosum* (a); *Eucheuma cottonii* (EC) (b); KCl-insoluble fraction (c); enyzmic resistant fraction (d); and KCl-soluble fraction (e) of EC.

proportion of methyl groups. Consequently, comparing the molecular weight (220 000) of ERF to that of EC it is apparent that EC contains a long block of iota-carrageenan and the case (a) in Fig. 2 is not possible. Only cases (b) or (c) remain. If two types of molecules exist in the extract (Fig. 2(c)) it must be possible to separate them.

The domains defined by temperature and counter ion concentration, in which kappa- and iota-carrageenans are in the random coil or the helical conformation, are different, and consequently the gelling ability of these polymers is different. In the presence of potassium there exists a zone (Fig. 6) where kappa-carrageenan adopts the helical conformation in a gel phase but where iota-carrageenan adopts a disordered conformation in a sol phase (Rochas & Rinaudo, 1984; Rochas, 1987). Consequently fractionation by KCl can separate the two molecules.

The soluble fraction in KCl (KSF) represents 11% (w/w) of EC. Its molecular weight and its intrinsic viscosity are 300 000 and 308 ml g^{-1} respectively. From the infrared spectrum and the ^{1}H NMR spectrum of KSF it appears that this fraction is a polysaccharide with a predomin-

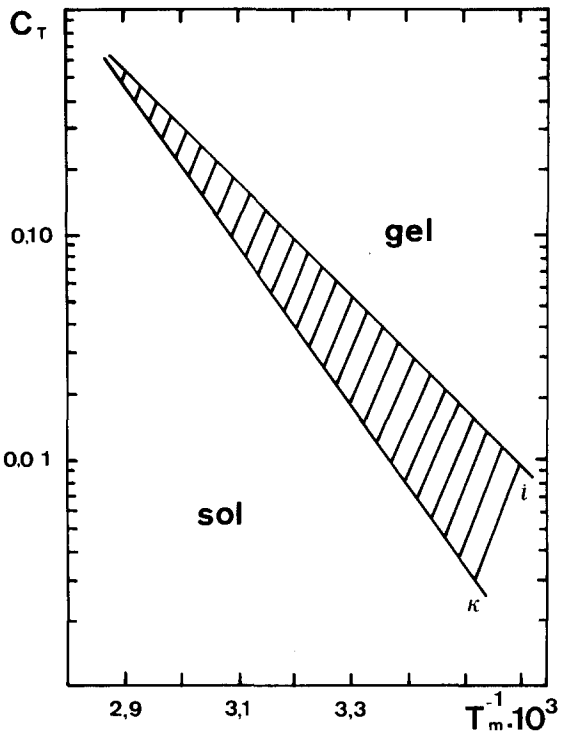

Fig. 6. Variation of the gelling temperature with the logarithm of the free potassium counter ion concentration for kappa- and iota-carrageenan.

ance of iota. The infrared spectrum and the ^1H spectrum of KSF are very close (Figs 3 and 5) to the spectra of the ERF fraction. In addition the sulfate content estimated from the infrared spectra (Rochas *et al.*, 1986) or microanalysis, and the methyl content estimated by ^1H NMR, are very similar for ERF and KSF (Table 1). Consequently we can assume that ERF and KSF represent the same material. Nevertheless if this polymer has an iota character from infrared and NMR data it appears that this iota polymer is partially unsulfated on the carbon 4 of the galactose and on the carbon 2 of the 3,6-anhydrogalactose. It must be noticed also that this polymer is highly methylated. This methylation occurs on position 6 of the galactose sugar as shown by gas chromatography of the alditol acetates obtained after acidic hydrolysis (see Experimental).

The KCl insoluble fraction (gel phase) is a polysaccharide with almost 100% pure kappa-carrageenan units. This fraction is fully hydrolyzed (98–99%) by the kappa-carrageenase. From its ^1H NMR spectra (Fig. 6), we notice that there are no more methylated galactan units or iota-carrageenan units.

TABLE 1
Sulfate and Methyl Contents of the Enzymic Resistant Fraction (ERF) and of the KCl-Soluble Fraction (KSF) Obtained by Different Techniques

	Sulfate on C-4 of D-galactose (infrared band at 845 cm^{-1})	*Sulfate on C-2 of 3,6 anhydrogalactose (infrared band at 805 cm^{-1})*	*Total sulfate[a] (infrared band at 1250 cm^{-1})*	*Total sulfate[a] (microanalysis)*	*Methyl[b] (^1H NMR)*
(KSF)	0·62	0·44	1·06	1·03	0·41
(ERF)	0·68	0·48	1·16	1·10	0·37

[a]Degree of substitution with sulfate per disaccharide repeat units.
[b]Degree of substitution with methyl per D-galactose unit.

Consequently we can conclude that the extract from *Eucheuma cottonii* is a mixture of polysaccharides with kappa-carrageenan as the major constituent and polymers with a large percentage of iota-carrageenan and methylated galactans as minor constituents.

The fractionation (0·03 M KCl, 20–22°C) used was also tested on extracts of *C. crispus*, on the alkaline-treated extract of *C. crispus* and on the alkaline-treated extract of *Gigartina stellata*. For these samples it was possible to separate a fraction (KCl soluble fraction) with iota-carrageenan as the major component and a fraction (KCl insoluble fraction) with kappa-carrageenan as the major component. The KCl insoluble fractions were 17, 64 and 40% of the total sample of *C. crispus*, alkaline-treated *C. crispus* and *G. stellata* respectively. The infrared spectra and the NMR spectra clearly show the iota or kappa nature of the different fractions separated in the alkaline-treated extract of *C. crispus* (Fig. 7). The fact that some iota structures exist in kappa-carrageenan sample sources probably explains the separation of two fractions with different gelling ability reported recently by Day *et al.* (1988) on a sample of crude kappa-carrageenan.

Relation between the structure and properties of the gels of *Eucheuma cottonii*

Considering that each original polysaccharide sample seems to consist of a mixture of polymers with different chemical structures, it was important to elucidate the role of heterogeneity in chemical structure on the physical properties of the gels.

In fact, the mechanical properties of a gel prepared from EC or from another algae is strongly dependent on the ratio of iota to kappa even if

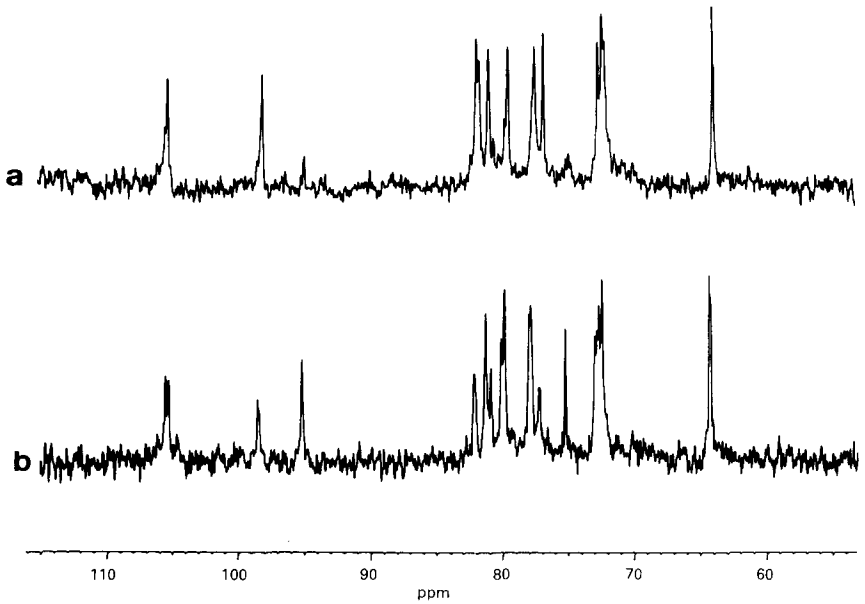

Fig. 7. ^{13}C NMR spectra of the KCl-soluble fraction (a) and the KCl-insoluble fraction (b) of the alkaline extract of *Chondrus crispus*.

the proportion of one of the two polysaccharides is low. These mechanical properties will depend on the relative composition of kappa and iota because the iota-carrageenan gel is very soft while kappa-carrageenan gel is brittle. To understand the role of each polysaccharide we prepared a mixture of EC and *E. spinosum* to represent blends from 100% kappa to 100% iota. To monitor the mechanical properties at small deformation and the ultimate properties, the Young's modulus and the yield stress were recorded as previously described (Rochas & Landry, 1988).

The modulus (Fig. 8) of the mixed gel decreased steadily from 100% kappa to 100% iota without any maximum. Assuming the additivity of the modulus of both polymers, we have tried to compute the modulus of the blends. For both polymers according to the total polymer concentration and the composition kappa–iota the modulus of each separated polymer is obtained from the linear relationship between the logarithm of the modulus and the concentration. The following relation was obtained (Rochas & Landry, 1988) for kappa-carrageenan in 0·25 M KCl at room temperature E (dyne/cm^2) = 7450 $C^{1.95}$ (g litre^{-1}). We obtained a relationship with about the same power for iota-carrageenan $E = 430$ $C^{1.97}$. No drastic differences occur (Fig. 8) between the calculated and the experimental values of the modulus. Due to the absence of any maximum on the modulus composition curve there is no strong evidence

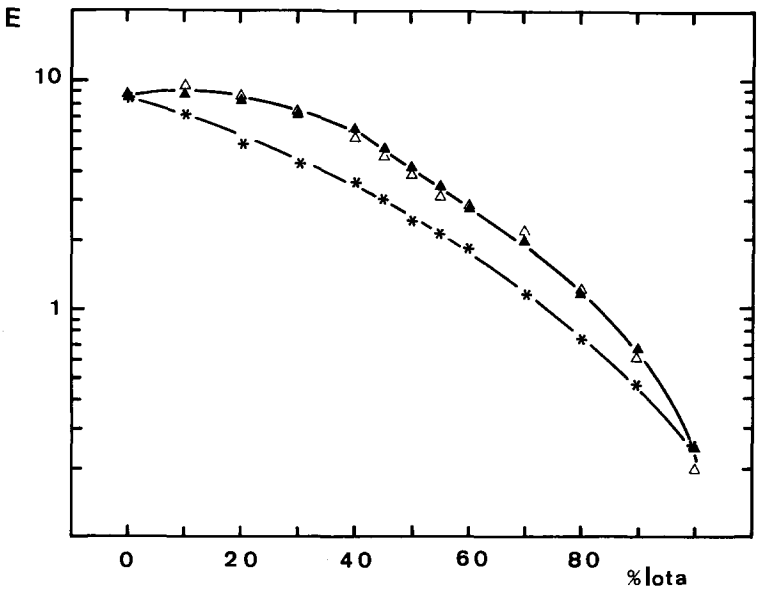

Fig. 8. Young's modulus E(10^5 dyne/cm^2) of the gels of the kappa-iota-carrageenans at room temperature, total polymer concentration 12 g litre^{-1}; KCl 0·25 M. △, Ageing time one night; ▲, aging time one week; ✱, computed values as described in text.

from these data alone for synergistic interactions. However the ultimate properties gave another view. The curve yield stress versus kappa-iota composition shows a marked maximum up to 76 N (Fig. 9) for a composition kappa-iota 50-50 even though the yield stress is 34 N and 3 N for pure kappa and iota respectively at a concentration of 12 g litre^{-1}. The relationship between the yield stress (F) and the polymer concentration (C) can be considered as a linear dependence for kappa-carrageenan (Rochas & Landry, 1988) for concentration higher than 3-5 g litre^{-1}. Also a linear relation is obtained for iota-carrageenan under the same conditions: $F(\mathrm{N}) = 1{\cdot}11\ C(\mathrm{g\ litre^{-1}}) - 6$. Consequently taking into account these relationships a yield stress of 16 N is calculated for a blend kappa-iota 50-50 at a total concentration of 12 g litre^{-1}. This computation is possible only because the deformation at the yield stress is identical for the two polymers and for the blend investigated. In this case the synergistic effect developed by a blend kappa-iota is very important because it corresponds to an increase of 375% in the yield stress. In addition the ageing of the blends (Figs 8, 9) does not drastically change the modulus and the yield stress of the blends investigated. Due to this synergistic effect we can postulate molecular interactions between iota and kappa chains. These specific interactions are clearly shown by

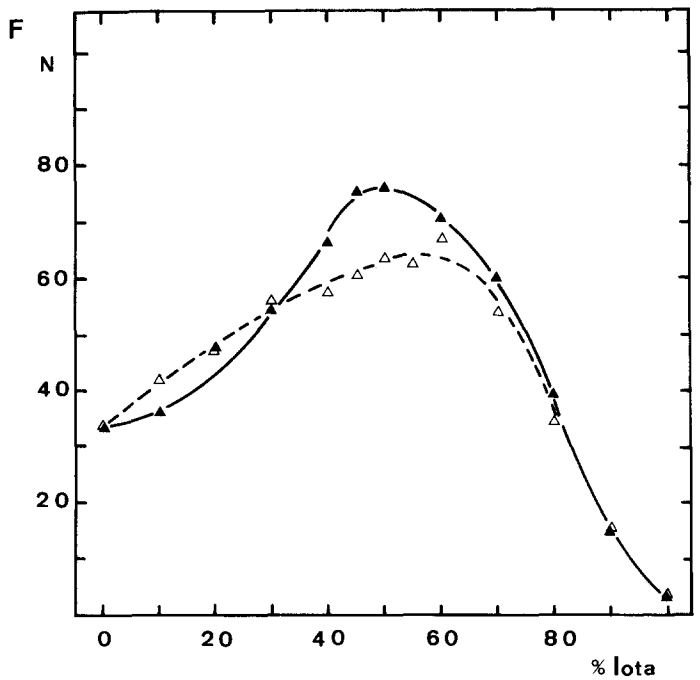

Fig. 9. Yield stress F_m (N) of the gels of the blends kappa-iota-carrageenans at room temperature, total polymer concentration 12 g litre^{-1}, KCl 0·25 M. △, Ageing time one night; ▲, ageing time one week.

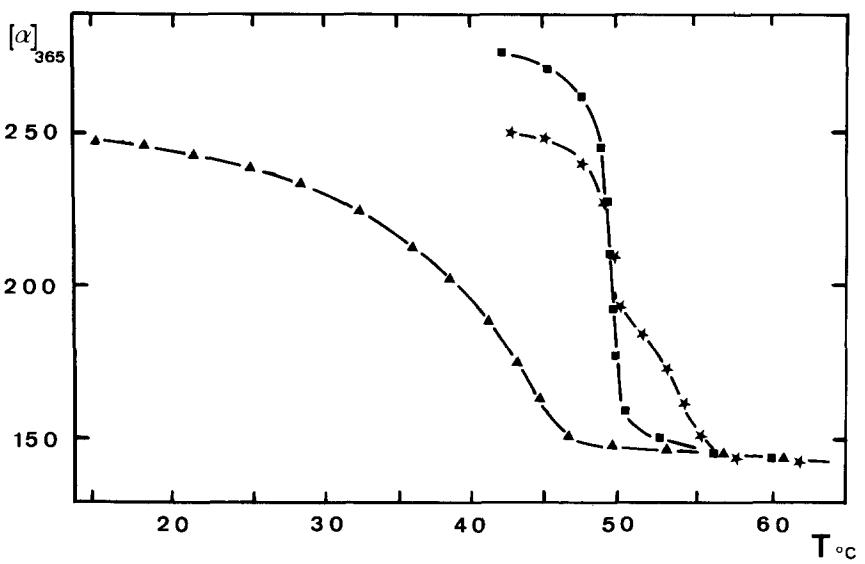

Fig. 10. Optical rotation temperature dependence of ■, kappa-carrageenan 0·5 g litre^{-1}; ▲, iota-carrageenan 0·5 g litre^{-1}; and of the blend kappa-iota-carrageenan ✶ (0·5 g litre^{-1} kappa and 0·5 g litre^{-1} iota).

the temperature dependence of the optical rotation of the blend (Fig. 10); in that case, for the kappa–iota mixture the onset of the transition is clearly different. The conformational transition starts at 47°C for iota-carrageenan, 50°C for kappa-carrageenan and 56°C for the blend. Consequently it seems that the number and/or the quality of the crosslinking of the network of mixed kappa–iota-carrageenan gels is enhanced in comparison with the pure forms of kappa- and iota-carrageenans. From these results it is possible to understand what happens when extracts of EC and pure kappa-carrageenan are compared. Completely different mechanical properties are obtained in both cases (Table 2). With regard to EC, the KCl insoluble fraction has a higher modulus. This is expected if we take into account the results shown in Fig. 7. *E. cottonii* contains about 11% of iota-carrageenan. The contribution of iota to the modulus is negligible compared with the contribution of kappa.

TABLE 2
Young's Modulus E (10^5 dyne/cm^2) and Yield Stress F(N) of the Extract of EC and the KCl-insoluble Fraction (KISF) of EC

		EC	*KISF*
E	5 g litre^{-1}	0·932	1·088
	10 g litre^{-1}	3·360	4·370
F	5 g litre^{-1}	8·92	5·33
	10 g litre^{-1}	28·85	15·99

The KCl insoluble fraction is more concentrated in kappa-carrageenan (11% more) and has a higher modulus (Table 2). We have shown (Fig. 9) that a small amount of iota increases the yield stress of the blend. Consequently we can understand that the fraction insoluble in KCl (KISF) obtained without iota-carrageenan gives a yield stress lower than that of EC.

From these results, it seems that a blend of these two polysaccharides could have interesting applications because there are synergistic interactions between them. These synergistic interactions explain the difference between mechanical properties in the gels obtained with polysaccharide from *E. cottonii*, containing a certain percentage of iota-carrageenan, and the gels obtained from *E. cottonii* purified by KCl fractionation, and free of iota-carrageenan.

These results show that it is important to accurately determine the structure of carrageenan to understand or to predict the properties of the crude samples.

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