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IMMUNOCHEMISTRY OF KAPPA-TYPE CARRAGEENANS FROM CERTAIN RED ALGAE

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ABSTRACT

Carrageenans from female and male gametophytic plants of the alga *Rhodoglossum californicum*, female plants of *Chondrus crispus* and *Gigartina pistillata*, and male plants of *Iridaea cordata* and a *Gigartina* species from San Francisco Bay were fractionated into potassium chloride-soluble and -insoluble components and were analysed chemically. An anti-κ-carrageenan, the reactivity of which is directed to κ-type structures (*i.e.*, 3-linked D-galactose 4-sulphate and 4-linked 3,6-anhydro-D-galactose residues) was used to analyse these carrageenans immunochemically. The potassium chloride-insoluble carrageenans from these species were found to be highly reactive κ-type carrageenans. The potassium chloride-soluble carrageenans were less reactive to anti-κ-carrageenan and, in addition, showed reactivity to an anti-λ-carrageenan preparation. The chemical and immunochemical data suggest that the potassium chloride-soluble carrageenans contain either λ- or μ-carrageenan, as a high proportion of the precursors to the 3,6-anhydro-D-galactose are 4-linked D-galactose 2,6-disulphate residues, and no increase in immunological reactivity to anti κ-carrageenan was observed upon alkali treatment.

INTRODUCTION

Carrageenans of the κ-type are composed ideally of alternating D-galactose 4-sulphate (Gal-4-S) and 3,6-anhydro-D-galactose (Gal-3,6-An) residues. This pattern may, however, be interrupted by the occurrence of D-galactose 6-sulphate (Gal-6-S), D-galactose 2,6-disulphate (Gal-2,6-diS), or non-sulphated residues, which Rees *et al.*[1] have termed "kinking residues". The net effect of these units on κ-type carrageenans is to render them soluble in potassium chloride solution (*i.e.*, if the proportion of "kinking residues" exceeds substantially the proportion of the 4-linked Gal-3,6-An residues[2]). McCandless *et al.*[3] have demonstrated that κ-carrageenan is restricted to the gametophytic stage of some algal species of the Gigartinales. Both potassium chloride-insoluble and -soluble components of carrageenans extracted from the gametophytic stage have been shown to exhibit κ-type characteristics[3]. It has been suggested that the KCl-soluble component contains the precursor (μ-type structure) to κ-carrageenan[4]. We have already demonstrated the usefulness of combining

TABLE I

CHEMICAL ANALYSIS OF CARRAGEENANS AND IMMUNOLOGICAL HOMOLOGY OF THE VARIOUS CARRAGEENANS TO *C. crispus* κ-TYPE CARRAGEENAN

Carrageenan source[a]	*Sulfate (%)*	*Gal-3,6-An*[b]			*Gal-2,6-diS (%)*	*Rate ppt.*[c]	*Equil.*[d]	*Ind. homol.*[e]
		Original	*After OH-BH_4*	*After IO_4^- and OH-BH_4*				
C. crispus (f.i.)	20.0	212	*f*	*f*		11.3	0.257	1.0
C. crispus (f.s.)	23.1	92	181.2	172.7	95.3	4.1	0.180	0.25
I. cordata (f.i.)	17.1	177	*f*	*f*		16.0	0.295	1.6
I. cordata (f.s.)	26.8	111	203.9	158.5	77.7	3.6	0.160	0.20
R. californicum (f.i.)	20.2	201	*f*	*f*		17.9	0.310	1.9
R. californicum (f.s.)	20.9	132	177.7	175.5	98.8	7.3	0.200	0.50
R. californicum (m.i.)	20.1	198	*f*	*f*		12.4	0.265	1.1
R. californicum (m.s.)	21.2	109	169.9	154.3	90.8	7.5	0.210	0.54
G. pistillata (f.i.)	12.1	192	*f*	*f*		10.5	0.260	0.94
G. pistillata (f.s.)	27.1	145	237.8	226.6	95.3	3.7	0.150	0.20
G. sp. (SFB) (m.i.)	18.0	189	*f*	*f*		14.4	0.280	1.4
G. sp. (SFB) (m.s.)	20.3	128	223.7	195.4	87.5	6.9	0.210	0.49

[a]Abbreviations: f., female; m., male; i., KCl-insoluble; s., KCl-soluble. [b]Ratio of Gal-3,6-An residues in μg to mg of carrageenan. [c]Rate of precipitation in Abs units · min^{-1} · 10^{-3} (± 10%). [d]Equilibrium (Absorbance units, ± 10%). [e]Index of homology = Test antigen (rate of ppt. × equilibrium Abs.)/reference antigen (rate of ppt. × equilibrium Abs.). [f]No significant change in the level of Gal-3,6-An residues after treatment.

immunochemical and chemical procedures in the elucidation of the carrageenan fine structure[5,6]. In this paper, we present a study of KCl-soluble and -insoluble κ-type carrageenans extracted from gametophytic phases of several species of red algae.

RESULTS

The i.r. spectra of the carrageenans used in this study showed marked similarities; all contained the general absorption band for carbohydrate ester sulphate at 1240–1250 cm^{-1}. The KCl-insoluble carrageenans from male and female algal species contained sharp, pronounced absorption bands at 936 and 840 cm^{-1}, indicating the presence of Gal-3,6-An and Gal-4-S residues, respectively. The KCl-soluble carrageenans of gametophytes also showed these κ-type-characteristic absorption bands. In general, the latter showed less pronounced absorption at 936 cm^{-1}, indicating a content of Gal-3,6-An lower than that of the KCl-insoluble carrageenans, and a broader band with maximum at 840 cm^{-1} attributed to the occurrence of Gal-6-S residues (absorption at 820 cm^{-1}). After alkaline sodium borohydride treatment (OH^-–BH_4), the content of Gal-3,6-An residues of these carrageenans increased (Table I), as shown by the increase in absorption at 936 cm^{-1}. Owing to the elimination of 6-sulphate groups in the process of conversion of Gal-6-S to Gal-3,6-An residues, a narrowing of the sulphate absorption band at 840 cm^{-1} was observed. The weak absorption at 805 cm^{-1} (Gal-3,6-An-2-S residues) observed in these carrageenans also increased in intensity upon OH^-–BH_4 treatment.

The content of Gal-3,6-An residues of the carrageenans before and after OH^-–BH_4 modification, the latter analysed before and after sodium periodate oxidation, and the sulphate content of the original polysaccharides are recorded in Table I. KCl-soluble and -insoluble carrageenans from various algae were treated with an

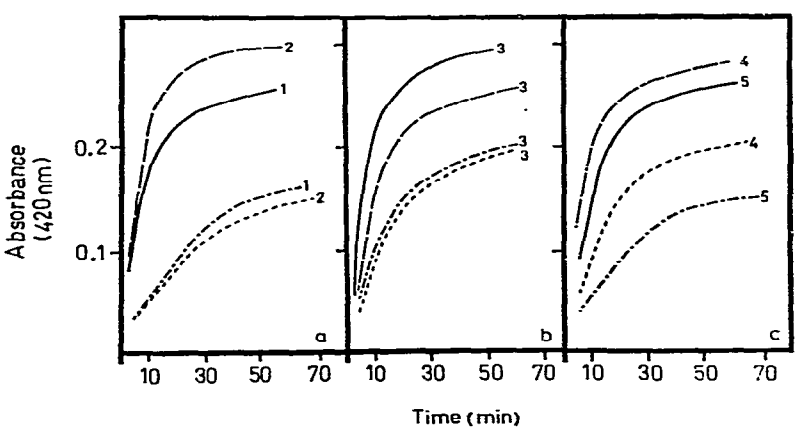

Fig. 1a–c. Immunochemical reactivity of *KCl insoluble* carrageenan from female (———), male (— — — —), and KCl-soluble carrageenans from female (— · — · —) and male (------) gametophytic algal plants of: (1) *C. crispus*, (2) *I. cordate*, (3) *R. californicum*, (4) *G.* species from San Francisco Bay, and (5) *G. pistillata*.

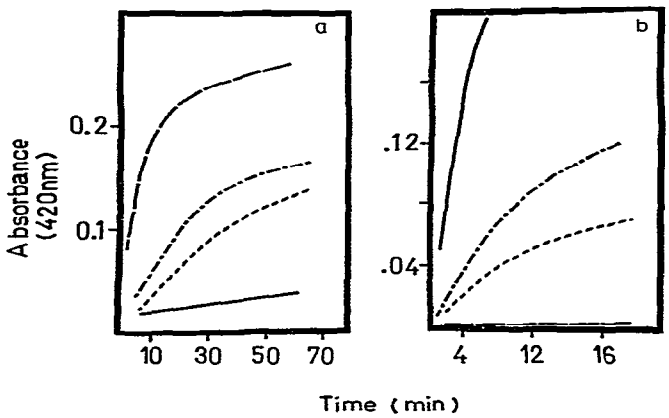

Fig. 2. Immunochemical reactivity of KCl-insoluble (-————-); KCl-soluble (—-—-—); KCl-soluble, OH–BH_4 modified (-----) carrageenan from female gametophytic; and KCl-soluble carrageenan from tetrasporic (———) algal plants of *C. crispus* to: (a) anti-κ-carrageenan[7] (G9-γ-477), (b) anti-λ-carrageenan[7] (G7-γ-477).

anti-κ-carrageenan, the reactivity of which is directed towards Gal-3,6-An associated with Gal-4-S residues[7]. Fig. 1 shows the reactivity of the KCl-soluble and -insoluble carrageenans from *Iridaea cordata* (male), *Rhodoglossum californicum* (male and female), a *Gigartina* species from San Francisco Bay (male), *Gigartina pistillata* (female), and *Chondrus crispus* (female). The indices of homology[8] of the various carrageenans to the homologous antigen (KCl-insoluble carrageenan from carposporic *Chondrus crispus*) are shown in Table II. The reactivity of the KCl-soluble carrageenan from *Chondrus crispus*, before and after OH^-–BH_4 treatment, to an anti-κ and an anti-λ carrageenan preparation is shown in Fig. 2a and 2b, respectively.

DISCUSSION

The i.r. spectra of the various carrageenans suggest that the KCl-soluble carrageenans from the gametophytic algal plants contain 4-linked Gal-6-S and less Gal-3,6-An residues than the respective KCl-insoluble carrageenans. After OH^-–BH_4 treatment of the KCl-soluble carrageenans, the major contribution to the sulphate group absorption region (800–850 cm^{-1}) is due to Gal-4-S residues with a maximum at 840 cm^{-1}. The weak absorption at 805 cm^{-1} is enhanced by OH^-–BH_4 treatment indicating that a proportion of the increase in the 936 cm^{-1} absorption band might be due to the appearance of Gal-3,6-An-2-S and not just to Gal-3,6-An residues. The content of 3,6-anhydride groups after OH^-–BH_4 treatment, analysed before and after sodium periodate oxidation (Table I), shows that this is in fact the case; 78 to 99% of the residues precursor for the 3,6-anhydride groups were resistant to $NaIO_4$ oxidation, suggesting that these residues are Gal-2,6-diS. Alkaline borohydride treatment of KCl-insoluble carrageenans caused little change in either the chemical, i.r., or immunological data.

The KCl-insoluble carrageenans reacted immunochemically better with an anti-κ-carrageenan antibody than did the KCl-soluble carrageenans. The indices of homology of the former compounds (Table I) ranged from 0.9 to 1.9. The KCl-soluble carrageenans were less reactive, the indices of homology ranging from 0.2 to 0.5. In these carrageenans, the lower reactivity may be correlated with higher contents of sulphate groups (Table I). The immunochemical variations encountered in the KCl-insoluble carrageenans cannot be accounted for by the slight quantitative variations in the chemical content of sulphate groups or Gal-3,6-An residues, although the presence of a 2-sulphate group on the 4-linked Gal-3,6-An residue has been shown to reduce reactivity with the anti-κ-carrageenan antibody. The homologous or reference antigen need not be the preparation that contains the highest proportion of κ-type determinants per equivalent. The ability of some κ-carrageenans to react differently from other κ-type carrageenans suggests the existence of cryptic antibody-binding sites in the less reactive carrageenans. These binding sites may be rendered cryptic by the molecular configuration of the particular polysaccharide, as determined by the internal organization of "kinking" residues and κ-type residues. The effect of "kinking residues" on secondary structure, and the effect of secondary and tertiary structure on the immunochemical reactivity of carrageenans to anti-carrageenans has already been discussed[1,5,6]. In addition, we have observed the occurrence of *O*-pyruvyl-D-galactose residues in some KCl-insoluble carrageenans from gametophytic algae[9]. Although the effect of such residues on carrageenan immunochemistry is unknown, they do play an important role in the immunochemical reactivity of bacterial polysaccharides[10].

The KCl-soluble carrageenans also show reactivity to an anti-λ-carrageenan (Fig. 2b shows the reactivity of the carrageenan from *C. crispus*). This indicates the presence of λ-like structures, probably associated with the higher levels[5,7] of Gal-6-S. Although the chemical data show that OH^-–BH_4 treatment renders the KCl-soluble carrageenans more similar to the KCl-insoluble κ-type carrageenan (*i.e.*, the content of 3,6-anhydride groups increases), the immunochemical reactivity to anti-κ-carrageenan remains low. Their reactivity to the anti-λ-carrageenan decreases, as might be predicted by the decrease in 6-sulphate group content[5]. The reactivity of the KCl-soluble carrageenans to anti-κ-carrageenan suggests that the preparation contains κ-type molecules that are soluble in KCl and possibly in the process of being synthesized. The chemical data (Table I) show that most of the residues precursor of 3,6-anhydride groups are Gal-2,6-di-S residues. The alkali-modified, KCl-soluble polysaccharides, therefore, either represent ι- or alkali-modified λ-carrageenan. Anti-κ-carrageenan reacts poorly with ι-carrageenan and not at all with alkali-modified λ-carrageenan[7]. McCandless *et al.*[3] showed that the OH^-–BH_4-treated, KCl-soluble carrageenan from *C. crispus* could be fractionated into KCl-soluble and -insoluble fractions. Hosford and McCandless[11] have shown the latter to be reactive with anti-κ-carrageenan serum, whereas the former showed little reactivity. The anti-κ-carrageenan serum used in that study, however, could not distinguish κ-carrageenan from either ι- or alkali-treated λ-carrageenan. A one-step chemical conversion of the

6-sulphate- to the 3,6-anhydride-containing residues is not sufficient to increase the immunochemical reactivity of the KCl-soluble carrageenan on alkali treatment. The theoretical μ-carrageenan as precursor to κ-carrageenan remains elusive, and it is suggested by both chemical and immunochemical data that removal of the 2-sulphate group from the 4-linked residue may be a necessary step in the synthesis of κ-carrageenan. Jackson[12] in this laboratory has also obtained evidence of Gal-3,6-An-2-S residues as intermediates in κ-carrageenan synthesis, from subfractionation of ^{35}S-labelled carrageenan from *C. crispus*.

EXPERIMENTAL

Materials. — Carrageenans were prepared and fractionated with potassium chloride as previously described by McCandless *et al.*[3].

Methods. — Alkaline borohydride and periodate oxidation were carried out as described by Lawson *et al.*[2]. Sulphate groups were determined by the method of Jones and Letham[13], 3,6-anhydro-D-galactose residues by the resorcinol method of Yaphe and Arsenault[14], and D-galactose 2,6-disulphate residues as suggested by Lawson *et al.*[2]. I.r. spectral analyses were carried out on carrageenan films formed by dissolving carrageenan (3 mg) with boiling water on silver chloride discs as described by Craigie and Leigh[15].

Antibody preparation and antigen–antibody reactions. — Anti-κ-carrageenan (G9-γ-477) and anti-λ-carrageenan (G7-γ-477) γG globulins were prepared as previously described[7], by injection of the specific carrageenans isolated from gametophytes and sporophytes of *Chondrus crispus*. Quantitative antigen-antibody reactions were carried out by the agarose-medium turbidimetric assay[8]; the antibody (250 μl) was treated with the antigen (25 μg) at 37° in a medium consisting of 0.1% agarose in 0.01M phosphate buffer in saline solution at pH 7.5 (3 ml). The rate of precipitation was recorded by an external recorder-readout connected to a Unicam 1800 Spectrophotometer set at a wavelength of 420 nm.

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