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THE IMMUNOCHEMISTRY OF λ-TYPE CARRAGEENANS FROM CERTAIN RED ALGAE*

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ABSTRACT

An antibody preparation directed against a structural feature associated with 6-sulphate groups was used to probe structural relations among certain λ-type carrageenans Immunochemical and chemical differences are described between the KCl-soluble carrageenans from tetrasporic algal plants of *Gigartina corymbifera*, *Gigartina* sp from San Francisco Bay, *Petrocelis middendorfii*, *Iridaea cordata*, *Rhodoglossum californicum*, and *Chondrus crispus* The differences in immunochemical reactivity of the *Gigartina* and *Petrocelis* carrageenans relative to the homologous antigen (*Chondrus crispus* λ-carrageenan) are attributed to the lower content of 6-sulphate groups on the 4-linked residues in the former carrageenans Both the immunochemical and chemical data suggest that the *Gigartina* and *Petrocelis* carrageenans are largely ξ-like in structure but do contain λ-like features The ir spectrum of the *Petrocelis* carrageenan differs from that of the *Gigartina* carrageenans The carrageenans from *I cordata* and *R californicum* differ to a lesser degree from *Chondrus crispus* λ-type carrageenan These differences cannot be accounted for by differences in the levels of 6-sulphate groups Some other structural feature, as yet unidentified, is responsible for the discrepancy in the immunochemical reactivity of these carrageenans to the anti-λ-carrageenan

INTRODUCTION

Several classes of carrageenan have been described These are defined[1,2] by their ester sulphate group content and position, as well as their solubility in KCl Within these classes of sulphated galactans, variations and deviations from ideal structure may occur[3] These variations have been exploited to study the conformational features and physical properties of carrageenans[4,5] As immunochemical techniques become more refined, these variations may prove useful for purposes of classification and taxonomy

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The demonstration that, in some members of the Gigartinales, κ-carrageenan is restricted to the gametophyte and λ-carrageenan to the sporophyte[6], has led to the preparation of purer κ- and λ-carrageenans In this paper, we present a quantitative immunochemical study of carrageenans extracted from tetrasporic stages of several species of algae The usefulness of such an approach in deducing immunostructural relationships as well as detecting gross or subtle chemical and structural variations is demonstrated

EXPERIMENTAL

Materials — KCl-soluble carrageenans from tetrasporic plants of *Chondrus crispus*, *Rhodoglossum californicum*, *Gigartina corymbifera*, *Gigartina* species (San Francisco Bay), *Petrocelis middendorfii* (*P franciscana*), and *Iridaea cordata* were extracted as described by McCandless *et al* [6]

Chemical modifications and analysis — A preparative, alkaline borohydride treatment was carried out according to a procedure described by Rees[1] Controlled alkaline $NaBH_4$ treatment was carried out by the same procedure, except that samples were removed at various times from 15 min to 10 h, and immediately precipitated with 2 vol of absolute 2-propanol The modified carrageenan was collected by centrifugation and washed with 80% 2-propanol until the samples were free of salt

Periodate oxidation was carried out as described by Rees[1] Determination of 3,6-anhydro-D-galactose was carried out by the resorcinol method of Yaphe and Arsenault[7]

Antibody preparation — Antibody (Ab) against κ-carrageenan from *C crispus* was raised in a goat by 4 injections administered on a biweekly basis, of carrageenan conjugated to methylated bovine serum albumin The serum was dialyzed against 10mM phosphate buffer (pH 5 2–5 4) at 5° to remove water-insoluble euglobin, which has a tendency to be precipitated nonspecifically by carrageenan[8] The gamma-globulin fraction was obtained by the slow addition of finely ground $(NH_4)_2SO_4$ to 50% saturation at room temperature The precipitate was centrifuged at 10 000 r p m for 20 min, resuspended in one half volume of 10mM phosphate buffered saline solution (PBS) (pH 7 5) The specific γG-globulin fraction was precipitated by the slow addition of finely ground $(NH_4)_2SO_4$ to 33% saturation at room temp collected by centrifugation, resuspended in one half the original serum volume of 10mM PBS (pH 7 5), and dialyzed against a large excess of the same buffer for 24 h at 5° The γG-globulin preparation was sterilized by Millipore filtration and stored at −70°

Quantitative antigen–antibody (Ag–Ab) reactions were carried out by the agarose medium turbidimetric assay[9], Ab (250 μl) was treated with Ag (25 μg) at 37° in a medium consisting of 0 1% agarose in 10mM PBS (pH 7 5, 3 ml) The rate of precipitation was recorded with an external recorder-readout attached to a Unicam 1800 spectrophotometer set at a wavelength of 420 nm

Infrared spectroscopy — I r spectral analysis was carried out on carrageenan films formed by dissolving carrageenan (3 mg) in boiling water on AgCl discs, as described by Craigie and Leigh[10]

RESULTS

Characteristics of the anti-λ-carrageenan — A commercial preparation of λ-carrageenan supplied by Marine Colloids, Inc, Springfield, NJ 07081, was subjected to controlled, alkaline borohydride treatment in order to obtain samples with varying amounts of D-galactose 6-sulphate residues Table I indicates the content of 3,6-anhydro-D-galactose residues of the samples An increased content of anhydro residues is concomitant with a decreased content of 6-sulphate groups as the anhydro residue is formed[1] by SN2 elimination of the 6-sulphate group The ability of each sample to precipitate anti-λ-carrageenan is shown in Fig 1 A marked decrease in reactivity is noted with increased modification

Immunochemical analysis of carrageenan — Equivalent amounts (25 μg) of various KCl-soluble carrageenans were treated with anti-λ-carrageenan Fig 2 shows the reactivity of KCl-soluble carrageenans from *C crispus*, *R californicum*, *I cordata G cornubifera*, *Gigartina* sp from San Francisco Bay and *P nuddendorfii* (*franciscana*) before and after alkaline $NaBH_4$ treatment The carrageenans from *I cordata* and *R californicum* show lower reactivity to the anti-λ-carrageenan than the homologous antigen (*i e*, *C crispus* carrageenan), whereas the *Gigartina* and *Petrocelis* show very low reactivity In all cases a further decrease in antigen–antibody reactivity was noted after alkaline borohydride treatment Table II shows the indices of homology (I H) of the various carrageenans calculated by the following formula described by DiNinno and McCandless[9] I H = experimental (precipitation rate × equilibrium absorbance)/reference (precipitation rate × equilibrium absorbance) where the reference sample is the homologous antigen

Chemical analysis of carrageenans — The i r spectra of the carrageenans show marked similarities in that all contain the usual carbohydrate ester sulphate band at 1250–1240 cm^{-1} The i r spectra of *C crispus R californicum* and *I cordata* carrageenans show a broad band between 850 and 800 cm^{-1} with a peak at 827 cm^{-1} After alkaline borohydride treatment the broad peak at 827 cm^{-1} was resolved into a peak at 805 cm^{-1} representing 3,6-anhydro-D-galactose 2-sulphate and a peak at 830 cm^{-1} for the *C crispus* KCl-soluble carrageenan with a concomitant appearance

TABLE I

SMELL SOLUTION AND 3,6-ANHYDRO-D-GALACTOSE CONTENT OF THE MARINE COLLOIDS λ-CARRAGEENAN AFTER CONTROLLED TREATMENT WITH ALKALINE SODIUM BOROHYDRIDE

Carrageenan sample no	*Duration of treatment (h)*	*3,6-Anhydro-D-galactose content (%)*
1	0	0 35
2	0 5	6 4
3	1	10 3
4	1 5	13 1
5	4	16 9
6	–	19 1

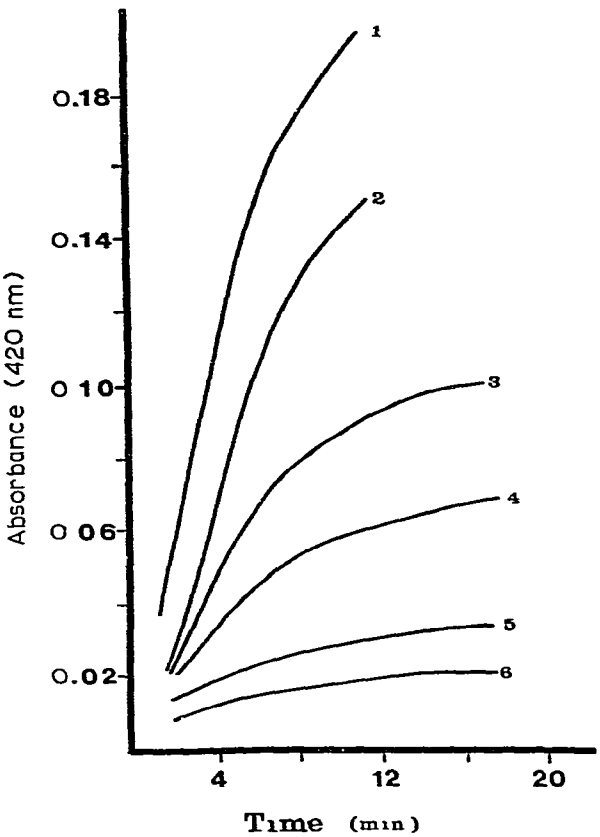

Fig 1 Turbidimetric assay in agarose[9] of carrageenans with various degrees of alkaline borohydride treatment See Table I for numbering of the samples

of the peak at 936 cm^{-1} due to the 3,6-anhydride group For *I cordata* and *R californicum* carrageenans, the band remained broad between 860 and 800 cm^{-1}, but the peak absorption was shifted to 835 cm^{-1}, indicating a change in the proportions of primary, secondary axial, and secondary equatorial ester sulphate groups, a peak at 936 cm^{-1} also appeared after alkaline borohydride treatment The spectra of the two *Gigartina* species show a narrow band with a peak at 830 cm^{-1}, which remains relatively unchanged after alkaline borohydride treatment The *P middendorfii* (*P franciscana*) carrageenan shows a broad peak between 850 and 800 cm^{-1} with a sharp peak at 830 cm^{-1} After alkaline borohydride treatment, the band remained broad with peaks at 845 and 835 cm^{-1}

The analysis of 3,6-anhydro-D-galactose content before and after alkaline borohydride treatment, the latter analyzed before and after $NaIO_4$ oxidation, are recorded in Table III The resistance of all these polymers to $NaIO_4$ oxidation demonstrates that all of the precursor units to the 3,6-anhydride residues are D-galactose 2,6-disulphate and not merely D-galactose 6-sulphate residues (Table III)

The *Gigartina* and *Petrocelis* carrageenans showed, after alkaline $NaBH_4$ treatment, contents of 3,6-anhydro residues lower than those of the *C crispus*, *R californicum*, and *I cordata* carrageenans, which indicates a lower content of 6-sulphate groups in the former carrageenans The *I cordata* and *R californicum* carrageenans have similar levels of 6-sulphate groups as *C crispus* carrageenan

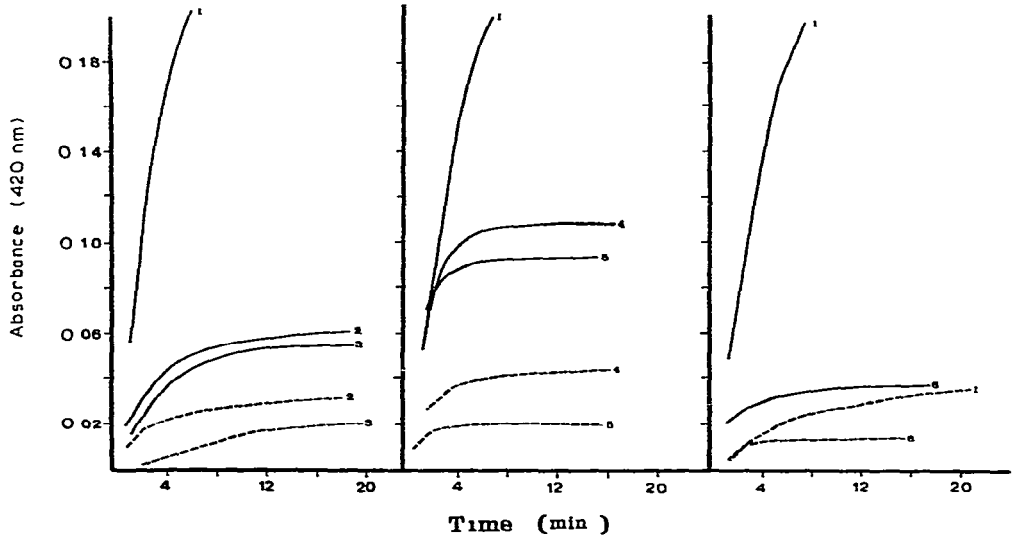

Fig 2 Turbidimetric assay in agarose[9] of carrageenans before (———) and after alkaline borohydride treatment (— — —) (1) *C crispus*, (2) *Gigartina* sp (San Francisco Bay) (3) *G corymbifera* (4) *R californicum* (5) *I cordata* and (6) *P middendorfii* (*P franciscana*)

TABLE II

IMMUNOCHEMICAL HOMOLOGY OF THE VARIOUS CARRAGEENANS TO *C crispus* λ-TYPE CARRAGEENAN

Source of carrageenan	*Carrageenan type*	*Equilibrium turbidity* (± 10%)	*Rate of precipitation* [(*Absorbance*/*min* 10^3) ± 10%]	*I H*[a]
C crispus	λ	0 240	46	1
	λ[b]	0 048	4 6	0 02
R californicum	λ	0 110	40	0 40
	λ[b]	0 044	12	0 05
I cordata	λ	0 098	45	0 40
	λ[b]	0 02	12	0 02
G corymbifera	λ	0 056	9 8	0 05
	λ[b]	0 020	2 0	0 01
Gigartina sp	λ	0 06	8 0	0 05
San Francisco Bay	λ[b]	0 03	3 4	0 01
P middendorfii	λ	0 04	12	0 05
(*franciscana*)	λ[b]	0 02	5 6	0 01

[a]See Results for definition [b]Alkaline $NaBH_4$-treated samples

TABLE III

CONTENT OF3,6-ANHYDRIDO RESIDUES OF λ-TYPE CARRAGEENANS

Source of λ-type carrageenan	*3 6-Anhydro residue (μg)/carrageenans (mg)*			*D-Galactose 2,6-disulphate precursor residues (%)*
	Untreated	*Treated with*		
		Alkaline borohydride	*Alkaline borohydride and periodate oxidation*	
C crispus	20	169 9	164 2	96 6
P middendorfii (*P franciscana*)	30	84 9	90 6	107
G corymbifera	40	96 3	90 6	94 1
Gigartina sp (San Francisco Bay)	55	107 6	101 9	94 7
I cordata	0 6	191 1	209 5	109 5
R californicum	0 13	164 0	162 3	98 8

DISCUSSION

Although the immunochemical method may be used to detect quantitative differences in certain specific chemical features of antigens[11], the actual antigen–antibody reaction is not determined solely by primary structures but represents a complex stereochemical interaction in which slight modifications or variations in primary, secondary, and tertiary structures may have pronounced effects on the interaction between antigen and antibody The reactivity of λ-carrageenans to anti-λ-carrageenan is greatly influenced by alkaline sodium borohydride treatment of the polymer (Fig 1) Whether the decreased reactivity to the antibody is due to the loss of 6-sulphate groups or to the secondary and tertiary conformational changes produced by the introduction of 3,6-anhydro residues cannot be determined precisely It is clearly indicated, however, that some structural feature associated with D-galactose 6-sulphate or possibly D-galactose 2,6-disulphate residues is involved in the antigen–antibody interaction The 6-sulphate group structural feature represents the major but not the sole determining factor since, even after exhaustive alkaline sodium borohydride treatment, the resulting polymer still shows some reactivity to the anti-λ-carrageenan

Immunochemically the potassium chloride-soluble carrageenans from *R californicum* and *I cordata* have closer homology to *C crispus* than the carrageenans from either of the *Gigartina* species and *P middendorfii* (*P franciscana*) The extremely low indices of homology of the *Gigartina* and *Petrocelis* carrageenans may be due to the lower content of D-galactose 2,6-disulphate residues and the ensuing conformational alteration The carrageenans from *I cordata* and *R californicum* show an index of homology of 0 4 Although the content of 6-sulphate groups in these polysaccharides is as high as that of *C crispus* carrageenan, and although they show an I H higher than those of the *Gigartina* and *Petrocelis* species carrageenans, some

other structural difference must be responsible for the I H value being < 1 This structural feature need not be associated with the 6-sulphate groups

Using immunodiffusion with an absorbed anti-λ-antisera and i r spectroscopy, Evelegh *et al* [12] and McCandless *et al* [13] have described qualitative differences in the same carrageenans These were interpreted as being due to the lack of 6-sulphate groups in the *Gigartina* carrageenans In the present study we have shown that the reactivity of an antibody preparation, processed as described herein, is directed toward some structural feature associated with 6-sulphate groups The close similarity of the carrageenans from the *Gigartina* and *Petrocelis* species discussed here to ξ-carrageenan from *G chamissoi*, *G canaliculata*, and *G atropurpurea* described by Penman and Rees[2] is indicated by the low I H relative to that of *C crispus* carrageenan, by the lower content of 6-sulphate groups and by the differences in the i r spectra As these carrageenans do contain enough 6-sulphate groups to produce 5% of 3 6-anhydride residues on alkaline sodium borohydride treatment, which is accompanied by a decrease in immunological reactivity to the anti-ν-carrageenan, these may in fact represent hybrid ξ-ν-carrageenan molecules, although the possibility that λ-carrageenan might occur as a separate minor component cannot as yet be ruled out The i r spectrum of the *Petrocelis* carrageenan after alkaline sodium borohydride treatment is unusual in that it contains an absorption band at 845 cm^{-1}

We conclude that immunochemistry, in conjunction with i r spectroscopy and chemical analysis, is useful in detecting and elucidating structural variations that occur in certain related classes of carrageenans

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