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DIFFERENCES IN CARRAGEENAN IN GAMETOPHYTES AND TETRASPOROPHYTES OF RED ALGAE

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Key Word Index—*Iridaea*; *Gigartina*; Rhodophyceae; red algae; carrageenan; cell wall.

Abstract—The yield and type of carrageenan in the marine red algae *Iridaea cordata*, *I. heterocarpa*, *I. lineare*, *Gigartina exasperata* and *G. papillata* was examined. The yield from the species studied ranged from 52 to 66%. In gametophytes, the carrageenan was 93% κ-carrageenan; in tetrasporophytes, the carrageenan was 95% λ-carrageenan.

INTRODUCTION

The marine red algae *Iridaea* and *Gigartina* (Rhodophyta, Gigartinales) are sources of the commercially valuable sulphated polysaccharide carrageenan. Carrageenan from these and other members of the Gigartinales has usually been fractionated into a KCl-insoluble fraction (κ-carrageenan) and KCl-soluble fraction (λ-carrageenan). κ-carrageenan has high gel strength and low viscosity while λ-carrageenan has low gel strength and high viscosity. These two types of carrageenan differ in their degree of sulphation and in their molecular structure [1]. It has long been known that different populations of carrageenan-producing algae differed in their κ/λ proportions and that some species contained only κ-carrageenan. It was suggested that such differences might be due to seasonal factors, or differences in habitat or age [2–4], but no successful correlation was established between carrageenan type and any of these factors. It was recently realised that the haploid gametophytes contain predominantly κ-carrageenan and diploid tetrasporophytes contain predominantly λ-carrageenan [5–8]. This paper details carrageenan yield and κ/λ-carrageenan proportions for *Iridaea cordata* (Turner) Bory, *I. heterocarpa* Postels and Ruprecht, *I. lineare* (Setchell and Gardner) Kylin, *I. cornucopiae* Postels and Ruprecht, *Gigartina exasperata* Harvey and Bailey, and *G. papillata* (C. Agardh) J. Agardh.

RESULTS AND DISCUSSION

The carrageenan yield from all samples of sufficient mass for analysis are given in Table 1 (see Table 4 for sources of plants). In all species examined, carrageenan was more than 50% of the dry matter of the plants. In *I. cordata*, the species most intensively investigated, carrageenan averaged 61 ± 5% of the dry wt. One sample yielded more than 70% carrageenan; the average for all the

Table 1. Carrageenan yield for species of *Iridaea* and *Gigartina*

Species	Yield* (% dry wt)	Samples	Determinations
I. cordata	61 ± 5	30	40
I. heterocarpa	57 ± 10	3	3
I. lineare	66	1	1
I. cornucopiae	52	1	1
G. exasperata	52 ± 7	6	6
G. papillata	52	1	1

* Mean ± s.d.

Table 2. Comparison of carrageenan yield in different types of *Iridaea cordata* and *Gigartina exasperata*

Species	Type	Yield* (% dry wt)	Samples	Determinations
I. cordata	Vegetative	62 ± 5	11	14
	Male	59 ± 4	4	6
	Female	65 ± 4	8	9
	Tetrasporic	64 ± 3	8	11
G. exasperata	Vegetative	51 ± 9	4	4
	Female	54	1	1
	Tetrasporic	55	1	1

* Mean ± s.d.

other species examined was 55 ± 8%. A comparison of the total yield of plants of different types (vegetative, male, female and tetrasporic) revealed no significant differences (Table 2).

A striking difference was discovered when the extracted carrageenan was fractioned into the κ and λ types: gametophytes have a very high proportion of κ-carrageenan and little or no λ-carrageenan while tetrasporophytes have little or no κ-carrageenan and a very high proportion of λ-carrageenan (Table 3). The species most thoroughly investigated was *I. cordata*; samples of the other five species of Gigartinales which were examined followed a similar pattern in their κ/λ-proportions.

In those species, *I. cordata* and *I. heterocarpa*, for which cytological observations of ploidy levels are available [9], the gametophytes are known to be haploid and the tetrasporophytes are known to be diploid. Thus the correlation of the haploid nuclear condition with the presence of κ-carrageenan and of the diploid nuclear condition with the presence of λ-carrageenan in *Iridaea* follows the same pattern that has been found in *Chondrus crispus*, a North Atlantic member of the Gigartinales [5, 6]. Except for *G. papillata*, all the species examined in this study have isomorphic gametophytic and tetrasporophytic phases. *G. papillata* is a member of the subgenus *Mastocarpus* which is characterized by the lack of a tetrasporophyte phase [10]. Recently, West [11] has demonstrated that *Gigartina agardhii* (Setchell and Gardner) is the gametophytic phase of the encrusting alga *Petrocelis franciscana* (Setchell and Gardner). It seems likely that *G. papillata*, which closely resembles *G. agardhii* in morphology and habitat, also has a heteromorphic life history and that its tetrasporophytic stage will be found to be an encrusting form, too. In addition *P. franciscana* which is known only as a tetrasporophyte has been shown to contain primarily λ-carrageenan (McCandless and West, personal communication). Thus it appears that carrageenan composition and life history phase can be correlated for all the Gigartinales which have been examined so far whether they are from the North Atlantic, the North Pacific or the South Pacific.

The fact that κ-carrageenan is characteristic of gametophytes (haploids) and λ-carrageenan is characteristic of tetrasporophytes (diploids) offers a relatively simple and reliable means of assessing

Table 3. Ratio of % κ-carrageenan to % λ-carrageenan in *Iridaea* and *Gigartina* species

Species	Vegetative or mixed samples	Male	Female	Tetrasporic
I. cordata	63/38	94/6	93/7	5/95
	(37)*	(4)	(5)	(3)
I. heterocarpa	—	94/6	94/6	0/100
I. lineare	—	91/9	87/13	24/76
I. cornucopiae	80/20	—	94/6	7/93
G. exasperata	98/2	—	96/4	0/100
G. papillata	—	—	93/7†	

* (s.d.).
† Males and females combined.

Table 4. Collection data of *Iridaea* and *Gigartina* species

Species	Site*	Dates
I. cordata	Minnesota Reef, San Juan Island	22–6–72
		18–7–72
		11–9–72
		12–5–73
		11–6–73
		15–4–73
		16–7–73
		18–9–73
		13–8–73
	Boulder Reef, Sinclair Island	23–7–72
	Middle Pt., Kitsap Peninsula	27–6–73
I. heterocarpa	Pt. Partridge, Whidbey Island	17–5–73
	Minnesota Reef, San Juan Island	13–8–73
I. lineare	Pt. of Arches, Olympic Peninsula	11–9–73
I. cornucopiae	Cape Flattery, Olympic Peninsula	10–9–73
G. exasperata	Middle Pt., Kitsap Peninsula	27–6–73
	Snug Harbor, San Juan Island	14–8–73
G. papillata	Pt. of Arches, Olympic Peninsula	11–9–73

* All sites are in Washington State.

the gametophyte/sporophyte ratios and ploidy levels of populations of carrageenan-producing algae especially when the plants are still vegetative and have not yet reached reproductive maturity. Such an assay should prove useful in studying the population biology of these algae to see if particular habitats favor the predominance of haploid plants or diploid plants. In commercial seaweed culture, it would be possible to select inoculum plants from populations such that a high proportion of one type of carrageenan or the other would be obtained in the cultivated crop. Furthermore since determination of carrageenan type can be done on individual plants or parts of individual plants, it should be feasible to select strains of these algae with particularly desirable characteristics, such as fast growth, by studying individual plants. The plants could then be grown to see which form of carrageenan was produced, and then vegetatively propagated for large-scale cultivation.

EXPERIMENTAL

Seaweed samples. Table 4 lists the species, dates, and places of collection of the samples. One to 10 kg (fr. wt) samples were sorted (vegetative, male, female, tetrasporic) then dried in the open air, in an electrically heated rotary dryer, or on racks next to a campfire.

Extraction. In determining extract yield, both a mild alkali and strong alkali extraction have been used; extract yields obtained by either method from subsamples were not significantly different (63 ± 5% mild alkali vs. 62 ± 4% strong alkali, $n = 10$). The extracts obtained by the strong alkali method were used for κ/λ fractionation. The strong alkali procedure differs from the mild only in the steps indicated by (strong alkali). Extraction was accomplished by adding 20 g clean, dry, pulverized seaweed sample to 0·7 l. of H_2O at 90–100° plus 10 ml 0·75 M NaOH (add 20 g sample to 500 ml cold 0·05 M $NaBH_4$ for 1 hr followed by addition of 200 ml 3 M NaOH plus 1 g $NaBH_4$); the mixture was heated for 1 hr with stirring then milled in a blender for 1 min. The blender was emptied and rinsed with 300 ml H_2O which was added to the paste which was stirred, covered, and held at 80° for 18 hr (the paste was brought to pH 8–9 with 3 M HoAc). The paste was mixed with 50 g filter aid (Celite 535), stirred 30 min and then about 500 ml was filtered in a preheated pressure filter. The filter cake was washed with 100 ml hot H_2O which was saved and added during the filtration of the remaining crude extract. All the filtrate and wash material was combined, weighed and then heated to 60°. The filtered extract was then poured with stirring into 1 vol. of 85% *iso*-PrOH in the ratio of 20 g filtrate:40 ml alcohol:1 ml 10% NaCl (omit NaCl in strong alkali procedure). After 15 min, the resulting coagulum was drained, squeezed dry and transferred to 300 ml of the 85% *iso*-PrOH soln for 15 min. After additional squeezing the coagulum was dried at 60° for 4 hr and ground to a powder when dry. Yield was determined gravimetrically based on the clean dry wt. of the sample.

Fractionation. κ/λ fractionation was accomplished by treating 8 g carrageenan (obtained by strong alkali extraction) with 800 ml of 2·5% KCl, stirring for 1 hr, then standing 18 hr, followed by addition of 20–50 g filter aid plus 1 hr stirring. The soln was then filtered through filter paper and the filter cake rinsed with 100 ml 2·5% KCl. The filtrate was added to 2·5 vol. of 85% *iso*-PrOH; the ppt. was λ-carrageenan which was washed once in 1 vol. of 85% *iso*-PrOH then dried 4–8 hr at 60°. The filter cake was dispersed in 800 ml hot H_2O and stirred 1 hr at 95°. The filtrate was added to 2·5 vol. of 85% *iso*-PrOH to precipitate κ-carrageenan which was washed once in 1 vol. of 85% *iso*-PrOH, then dried 4–8 hr at 60° in a well-ventilated oven to constant weight. Proportions were determined gravimetrically [12–15].

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REFERENCES

1. Percival, E. and McDowell, R. H. (1967) *Chemistry and Enzymology of Marine Algal Polysaccharides*, Academic Press, London.
2. Black, W. A. P., Blakemore, R. W., Colquhoun, J. A. and Dewar, E. T. (1965) *J. Sci. Food Agr.* **16,** 573.
3. Fuller, S. W. (1971) Ph.D. Thesis, Univ. of New Hampshire, Durham.
4. Rigney, J. A. (1971) *Progr. Rep.* **39,** Ind. Dev. Branch, Dept. Fish and Forestry, Ottawa.
5. Chen, L. C.-M., McLachlan, J., Neish, A. C. and Shacklock, P. F. (1973) *J. Mar. Biol. Ass. U.K.* **53,** 11.
6. McCandless, E. L., Craigie, J. S. and Walter, J. A. (1973) *Planta (Berl.)* **112,** 201.
7. Pickmere, S. E., Parsons, M. J. and Bailey, R. W. (1973) *Phytochemistry* **12,** 2441.
8. Waaland, J. R. (1973) *J. Phycol. (Suppl.)*, **9,** 12.
9. Fralick, J. E. and Cole, K. (1973) *Sycsis* **6,** 271.
10. Setchell, W. A. and Gardner, N. L. (1933) *Univ. Calif. Publs. Bot.* **17,** 255.
11. West, J. A. (1972) *Br. Phycol. J.* **7,** 299.
12. Rees, D. A. (1963) *J. Chem. Soc.* 1821.
13. Stancioff, D. J. and Stanley, N. F. (1969) *Proc. Intl. Seaweed Symp.* **6,** 595.
14. Smith, D. B. and Cook, W. H. (1953) *Arch. Biochem. Biophys.* **45,** 232.
15. Smith, D. B., Cook, W. H. and Neal, J. L. (1954) *Arch. Biochem. Biophys.* **53,** 192.