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Note

Eucheuma spinosum polysaccharides having different, average molecular weights

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Polysaccharidic material extracted with water from *Eucheuma spinosum* appears to consist of two different components containing low and high sulfate contents. The main component, which is readily extracted, is of a higher sulfate content than the minor component[1,2]. Evidence is presented to show that these two polysaccharides are of different, average molecular size.

EXPERIMENTAL

Extraction of polysaccharides. — The polysaccharides were extracted with water at 4° and at 100°, and the extracts were dialyzed and freeze-dried as previously described[1,2].

Preparation of Sepharose columns, and separation of polysaccharides from the columns. — Sepharoses 6B, 4B, and 2B were used. The procedure for the preparation of these gels, the method for packing the columns, and the separation of the polysaccharides from the columns were as previously described[1,2].

Elemental analysis. — Carbon, hydrogen, and sulfur were determined as previously described[1,2].

RESULTS AND DISCUSSION

Earlier work on the water-extractable polysaccharides from *Eucheuma spinosum* had indicated that there are two distinct polysaccharides[1,2]. The earlier fraction contains the polysaccharide having the higher sulfate content and an average molecular weight[1,3] of ~1.5 million. Table I shows that this is the major component, and that the polysaccharide having the lower sulfate content is the minor component. The polysaccharide having the lower sulfate content is also shown to be extracted after the polysaccharide having the higher sulfate content has been removed.

In an attempt to increase the yield of the minor component, subsequent extractions at 100° were made. The results of the elemental analyses shown in Table I,

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TABLE I

CARBON, HYDROGEN, AND SULFUR CONTENTS[a] OF *Eucheuma* POLYSACCHARIDES EXTRACTED SUCCESSIVELY AT 4° AND 100°

Fraction	*Dry wt. of seaweed (%)*	*C (μmol)*	*H (μmol)*	*S (μmol)*	*C/H*	*C/S*
Extracted at 4°						
1	76	2.76	4.87	0.243	0.57	11.36
2	3	2.88	5.35	0.226	0.54	12.74
3	1	3.99	7.51	0.0656	0.53	60.82
4	1	4.00	7.65	0.0746	0.52	53.62
Extracted at 100°						
5	0.75	3.66	7.29	0.0481	0.50	76.09
6	0.5%	3.68	7.55	0.0625	0.49	58.88

[a]Results are the mean of 3 experiments.

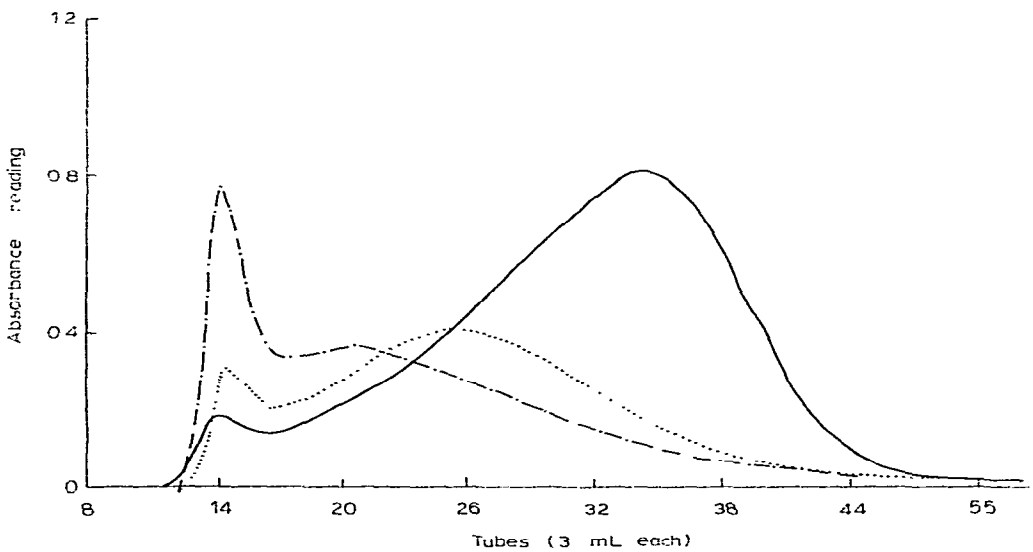

Fig. 1. Separation of *Eucheuma spinosum* polysaccharides on columns (1.5 × 80 cm) of Sepharose-4B. [Polysaccharide solution (0.2%, 2 mL) on column eluted with 0.3% NaCl, and 3mL of eluate collected. Key: ———, high-sulfate polysaccharide; —·—, low-sulfate polysaccharide; --------, Blue Dextran.]

and results obtained earlier[2], indicated that, in each experiment, the first two extracts were similar, and could therefore be pooled for dialyzing and freeze-drying, and the later extracts were also pooled for similar treatment. The freeze-dried material was dissolved in water, and a 0.2% solution (2 mL) was separated on Sepharose columns, and the elution patterns of the two polysaccharides compared. Both polysaccharides were found to be excluded from columns of Sepharose-6B gel, with exclusion limits of

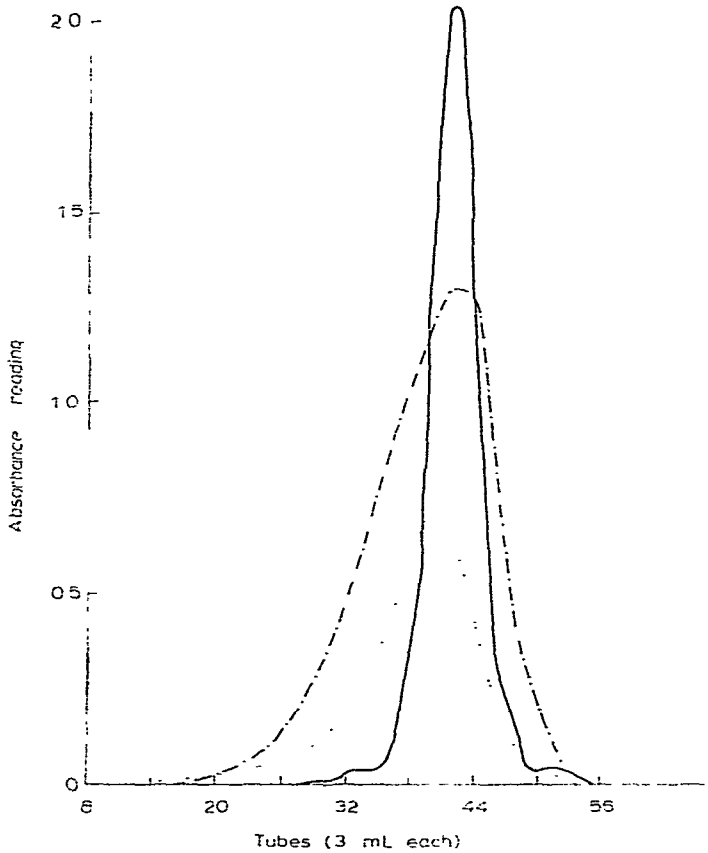

Fig. 2. Separation of *Eucheuma spinosum* polysaccharides on columns of Sepharose-2B. [Column size and conditions as described for Fig. 1. Key: ———, high-sulfate polysaccharide; — — —, low-sulfate polysaccharide; ·····, Blue Dextran.]

a molecular weight of 1 million for polysaccharides. Fig. 1 shows their separation on columns of Sepharose-4B. The polysaccharide having the lower sulfate content had a large proportion of molecules excluded by columns of Sepharose-4B, with exclusion limits of a molecular weight of 5 million, whereas the other polysaccharide could be almost completely separated by these columns. Thus, the polysaccharide component having the lower sulfate content appears to have the greater, average molecular weight. This difference was not, however, sufficiently great to be clearly seen when the two polysaccharides were separated on columns of Sepharose-2B having exclusion limits of a molecular weight of 20 million. Their elution patterns are shown in Fig. 2, together with that of the marker of Dextran Blue 2000 having $\overline{M}_w$ 2 million. It should, however, be noted that the polysaccharide of lower sulfate content had molecules constituting a wider range of molecular size than that of the other, and its elution pattern appeared to be rather similar to that of the Dextran Blue marker having an average molecular size of 2 million. Thus, its average molecular

size is probably larger than that of the other polysaccharide, whose elution pattern had previously been shown[3] to correspond rather closely to that of a Dextran T2000 fraction, with a narrow and well defined molecular weight of $\overline{M}_w$ 1.5 million.

It was, therefore, concluded that the polysaccharidic material extractable with water from *Eucheuma spinosum* consists of at least two polysaccharide species, a major and a minor component, differing in sulfate content and average molecular size. The major component is the polysaccharide having the higher sulfate content and lower $\overline{M}_w$ (1.5 million), and hence, it is the more readily extractable with water. The minor component, which constitutes only $\sim 4\%$ of the total, water-extractable polysaccharides (see Table I) has a lower sulfate content, and a greater, average molecular size (~ 2 million). This polysaccharide, which has molecules covering a wider range of molecular sizes, had previously been shown to have a higher glucose content[1,2], although galactose and 3,6-anhydrogalactose are the main sugar components of both polysaccharides[1].

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