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THE MOLECULAR WEIGHT AND VISCOSITY OF THE WATER-SOLUBLE POLYSACCHARIDE(S) FROM *Eucheuma spinosum*

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ABSTRACT

Polysaccharidic material extracted from *Eucheuma spinosum* with water at 4° was separated on columns of Sepharose-6B, -4B, and -2B. The average molecular weight was found to lie between 1.5 and 2 million (by comparison with that of a dextran fraction of narrow, and well defined, weight-range). A sample of κ-carrageenan was found to consist of much larger molecules having a molecular weight of 20 million or more. Viscosity measurements made on the *Eucheuma* polysaccharide(s) and κ-carrageenan sample showed their relative molecular weights to agree closely with those obtained by the gel-filtration studies, indicating that these polysaccharides could be of similar, polymeric species. The intrinsic viscosities of the *Eucheuma spinosum* polysaccharides were, however, much greater than that of the dextran of similar, average molecular weight.

INTRODUCTION

A study of the components of the red seaweed *Eucheuma spinosum* had previously been made with a view to improving its commercial value[1,2]. Carbohydrate was found to be the chief component, accounting for >70% of the dry weight, and to consist mainly of polysaccharide(s) extractable with water under mild conditions. The main sugar units of the polysaccharide(s) were found to be galactose and 3,6-anhydrogalactose in the ratio of 3:2.

We now report that the polysaccharidic material extracted from dried *Eucheuma spinosum* plants with water at 4° has an average molecular weight of 1.5–2 million and an intrinsic viscosity of 76.92. A sample of κ-carrageenan obtained from Marine Colloids Inc., U.S.A., was found to have a much greater, average molecular weight and intrinsic viscosity than the *Eucheuma* polysaccharide(s). From these observations, it is suggested that the difference in molecular size could also account for the difference in the precipitability of these two polysaccharides in 0.25M KCl solutions, in spite of their chemical and structural similarities[3].

EXPERIMENTAL

Extraction of polysaccharide(s). — The polysaccharide was extracted from

Eucheuma spinosum with water at 4°, and the extract was dialyzed and freeze-dried as previously described[1,2].

Preparation of Sepharose columns, and separation of polysaccharide from the columns. — Sepharoses 6B, 4B, and 2B were used. Before use, each gel was washed thoroughly with 0.3% NaCl until free from azide. The swollen gel was then packed into a column (80 × 1.5 cm) at 4°, and a 0.2% (w/v) solution of the polysaccharide (2 mL) was applied to the column, and eluted with 0.3% NaCl. A 2-mL portion of each of the 3-mL fractions collected was used for reaction with 4 mL of 0.2% anthrone in conc. H_2SO_4. The absorption was measured at[4] 625 nm. Blue Dextran 2000 (average molecular weight, 2 million) was eluted from each of the gel columns, either prior to, or after, the polysaccharide studied, by application of 2 mL of 0.2% (w/v) Blue Dextran 2000 to each column and measurement of the absorbance, at 264 nm, of each of the 3-mL eluates collected.

Viscosity measurements. — The relative viscosity of the polysaccharide was measured with a Beckman Low-shear Rotary Viscometer at 20 ±0.1° by using aqueous solutions of the polysaccharide of concentrations 0.01 to 0.05 g per 100 mL. From the relative viscosity at each concentration, the specific viscosity was calculated, and this was used for the determination of the intrinsic viscosity of the polysaccharide.

The intrinsic viscosity of the polysaccharide was obtained by using an equation given by Fuoss and Strauss[5] for flexible polyelectrolytes.

Intrinsic viscosity = [specific viscosity × $(1 + B\sqrt{C})]/C$, where B = a constant and C = the concentration in g per 100 mL of solution.

RESULTS

The polysaccharide extract from *Eucheuma spinosum*, consisting[1,2] of galactose and 3,6-anhydrogalactose units in the molar ratio of 1:0.63, was separated on Sepharose columns and compared with a sample of κ-carrageenan having[1,2] a galactose:3,6-anhydrogalactose ratio of 1:0.77. Both polysaccharides were found to be excluded from columns of Sepharose-6B gel with exclusion limits of 1 million molecular weight for polysaccharides. The κ-carrageenan sample was also totally excluded from columns of Sepharose-4B gel, which shows that its molecular weight exceeded 5 million. The *Eucheuma* polysaccharide material was, however, partially separated by these columns. Fig. 1 shows its elution pattern, together with that of Dextran Blue 2000 having $\overline{M}_w$ 2 million (used as a marker) and that of Dextran T2000, a fraction of narrow and well defined molecular weight of $\overline{M}_w$ 1.5 million, and a limiting viscosity number of 0.68. From these experiments, the *Eucheuma* polysaccharide appears to have an average molecular weight of 1.5 to 2 million.

Fig. 2 shows that the *Eucheuma* polysaccharide was completely separated by columns of Sepharose-2B having exclusion limits of 20 million molecular weight, whereas the κ-carrageenan sample was only partially separated. The difference in molecular size between these two polysaccharides is clearly seen from their elution patterns on the Sepharose-2B columns.

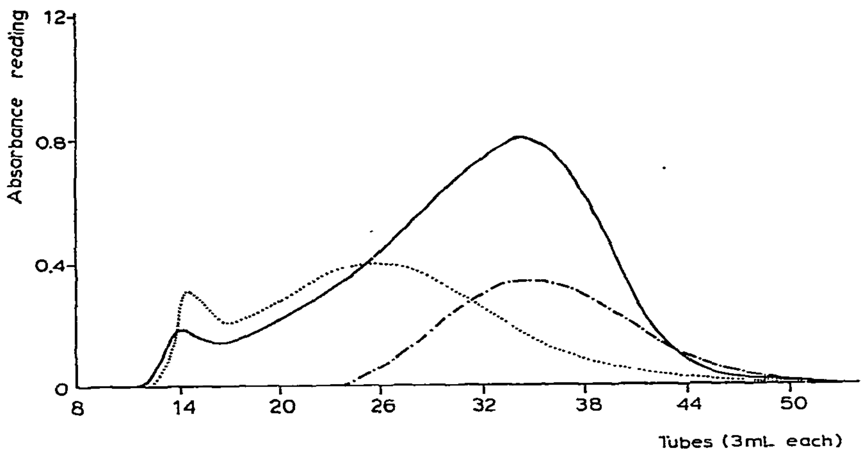

Fig. 1. Separation of *Eucheuma* polysaccharide(s) and Dextran T2000 ($\bar{M}_w$ 1.5 million) on Sepharose-4B columns (1.5 × 80 cm). [Polysaccharide solution (0.2%, 2 mL) on column eluted with 0.3% NaCl, and 3 mL of eluate collected. Key: ——, *Eucheuma* polysaccharide; ····, Blue Dextran; —·—, Dextran T2000 ($\bar{M}_w$ 1.5 million).]

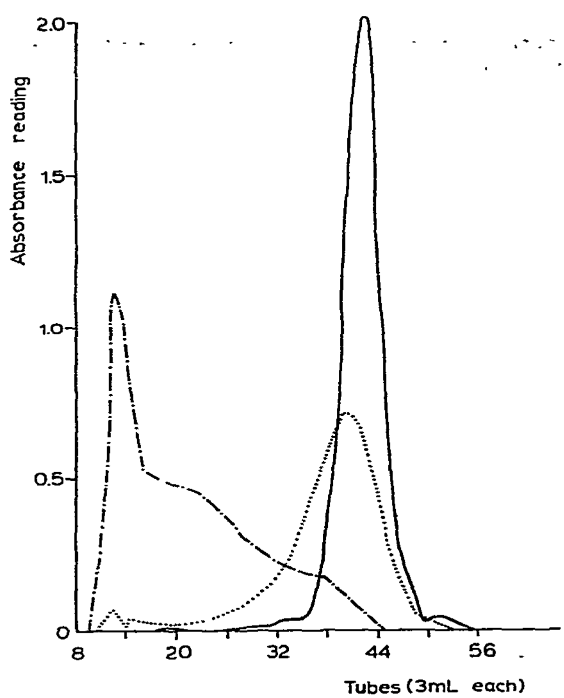

Fig. 2. Separation of polysaccharides from *Eucheuma spinosum* and κ-carrageenan sample (from Marine Colloids, Inc.) on Sepharose-2B column. [Column size and conditions as described for Fig. 1. Key: —·—, κ-carrageenan; ——, *Eucheuma* polysaccharide(s); ····, Blue Dextran.]

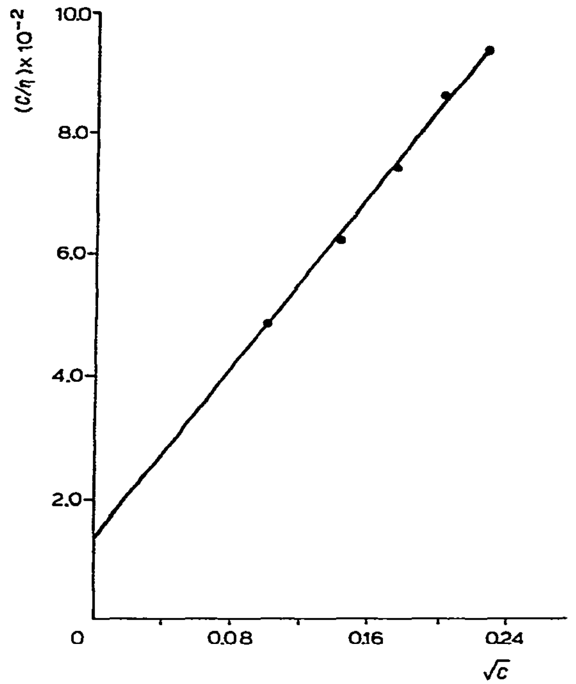

Fig. 3. The specific viscosity of the initial extracts of *Eucheuma spinosum* polysaccharide(s) at various concentrations. [Intercept at $\sqrt{C} = 0$ gave intrinsic viscosity^{-1} as 0.013.]

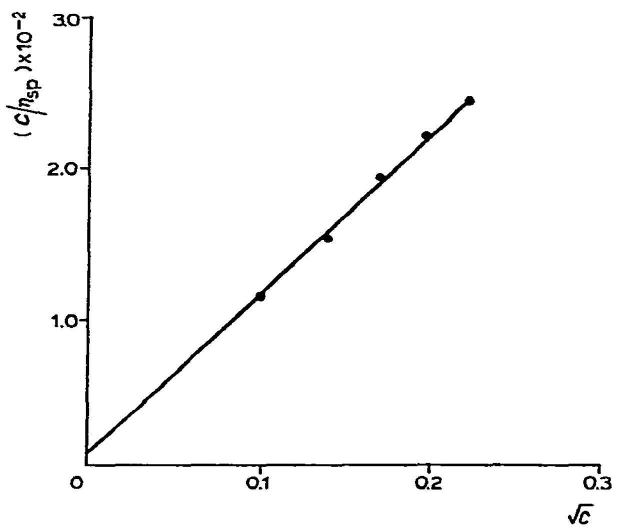

Fig. 4. The specific viscosity of the κ-carrageenan sample at various concentrations. [Intercept at $\sqrt{C} = 0$ gave intrinsic viscosity^{-1} as 0.001.]

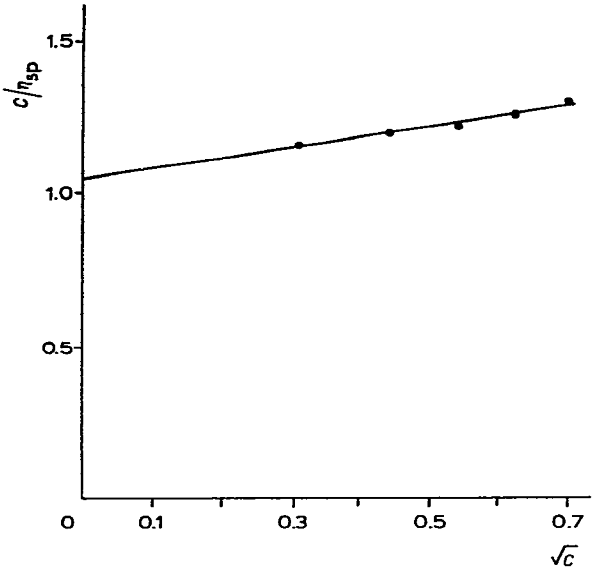

Fig. 5. The specific viscosity of Dextran T2000 ($\overline{M}_w$ 1.5 million) at various concentrations. [Intercept at $\sqrt{C} = 0$ gave intrinsic viscosity^{-1} as 1.05.]

The specific viscosities of the *Eucheuma* polysaccharide(s), κ-carrageenan sample, and Dextran T2000 ($\overline{M}_w$ 1.5 million) at various concentrations are respectively shown in Figs. 3, 4, and 5. From these graphs, plotted according to Tanford[6], the intrinsic viscosity of each polysaccharide was obtained. The intrinsic viscosity of the *Eucheuma* polysaccharide was 76.92, and that of the κ-carrageenan sample, 1000. The intrinsic viscosity of the Dextran T2000 fraction having $\overline{M}_w$ 1.5 million, and an average molecular weight approximately similar to that of the *Eucheuma* polysaccharide, was 0.95.

DISCUSSION

It has been reported[7] that the molecular size of κ-carrageenan as determined by ultracentrifugation and viscosimetric methods ranged from 330,000 to 790,000. From the present study, the κ-carrageenan sample (obtained from Marine Colloids, Inc., U.S.A.) appeared to have molecules exceeding this range of molecular weights, as determined by gel-filtration methods. However, marine algal polysaccharides are known[3] to have molecular weights ranging from 5,000 to several million. The *Eucheuma* polysaccharide appears to have $\overline{M}_w$ of 1.5 to 2 million, and we have been unable to find in the literature any reports with which to compare our results. Although the method of gel filtration that we have used in the determination of molecular size may provide only an approximate value, it does, however, give an indication of size, especially in relation to that of the κ-carrageenan polysaccharide.

The agreement between the results obtained for the relative, average molecular weights of these two polysaccharides through viscosity measurements and gel-filtration methods confirms, however, the conclusions of Black *et al.*[8] that the two polysaccharides are of similar, if not identical, polymeric species.

Anderson and co-workers[9] showed that the *Eucheuma* polysaccharide is readily distinguishable from κ-carrageenan by the presence of 3,6-anhydro-D-galactose 2-sulfate residues. There are also differences in the ratios of various constituents[1]. The fact that the *Eucheuma* polysaccharide consists of molecules of much smaller, average molecular size than those of the κ-carrageenan sample could, however, also be responsible for the observation that the *Eucheuma* polysaccharide is less readily precipitated (from solution) by dilute KCl than κ-carrageenan.

The difference in intrinsic viscosity between the *Eucheuma* polysaccharide and the Dextran T2000 sample having a similar, average molecular weight also suggests that the *Eucheuma* polysaccharide is unbranched, or a much less branched polymer than the Dextran, as branching in the polymer chain results[10] in decreased, intrinsic viscosity.

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