Carbohydrate Research, 59 (1977) 531–537

FUCOSE-CONTAINING POLYSACCHARIDES IN THE BROWN ALGAE *Ascophyllum nodosum* AND *Fucus vesiculosus*

DARRELL G. MEDCALF
Department of Chemistry, University of Puget Sound, Tacoma, Washington 98416 (U. S. A.)
AND BJØRN LARSEN
Institute of Marine Biochemistry, University of Trondheim, Trondheim (Norway)
(Received September 20th, 1976; accepted for publication in revised form, February 25th, 1977)

ABSTRACT

The fucose-containing, sulfated polysaccharides from *Ascophyllum nodosum* and *Fucus vesiculosus* were isolated by extraction with water adjusted to pH 2. Pure fractions were carefully separated by fractional precipitation with ethanol from aqueous solutions containing magnesium or calcium chloride. Progress in the fractionation efforts and purity of the fractions isolated were established by free-boundary and cellulose acetate electrophoresis. Ascophyllan, two "complexes", and a galactofucan were isolated from *A. nodosum*. An ascophyllan-like fraction, and a "complex" were isolated from *F. vesiculosus*. Mild, acid hydrolysis (0.02M hydrochloric acid, 1 h, 80°) converted each of the "complexes" into an electrophoretically faster-moving and a slower-moving component. The "complex" from *F. vesiculosus* comprised a greater proportion of the extract than did the two "complexes" from *A. nodosum*. In addition, the *Fucus* "complex" was richer in fucose*. However, the data suggest that neither species contains a pure fucan sulfate in the native state.

INTRODUCTION

Brown algae contain a complex array of fucose-containing, sulfated polysaccharides[1–4], which have proven difficult to characterize definitively. Larsen *et al.*[1,2] identified ascophyllan (a xylofucoglucuronan) plus several "complexes" that appeared to consist of protein, ascophyllan, and fucan. Mian and Percival[4] concluded that these polymers comprised a spectrum from high uronic acid, low sulfate-containing polymers to a fucan sulfate that was relatively pure. Recently, sulfated fucans have been studied with regard to their role in the early development of *Fucus* embryos[5–7]. The possible role of these polymers in the developmental biology of the algae has reemphasized the need for their definitive characterization.

---

*Enantiomeric identities of the component sugars reported in this and the following paper were not established by any direct method.

The present study has reexamined the fucose-containing, sulfated polysaccharides from two well studied species of brown algae, namely *Ascophyllum nodosum* and *Fucus vesiculosus*, with the major emphasis on the former. By using electrophoretic mobility as a criterion of purity, a definitive group of fucose-containing, polysaccharide fractions has been isolated and partially characterized.

RESULTS AND DISCUSSION

Initial, preliminary extractions were effected with both water acidified to pH 2 and 0.1M ethylenedinitrilo(tetraacetic acid) (EDTA) at pH 7.5. Free-boundary electrophoresis at pH 2 and at pH 7, and cellulose acetate electrophoresis at pH 7.5, indicated that both extractions gave essentially the same results. Therefore, subsequent work employed material extracted with dilute acid at room temperature.

The fractionation technique used by Larsen, *et al.*[1,2] was applied, with electrophoresis on cellulose acetate to monitor the results in small-scale experiments. Optimum conditions were used for preparative-scale fractionations and the fractions were characterized by both free-boundary (pH 2) and cellulose acetate (pH 7.5) electrophoresis. Purified fractions were refractionated until free-boundary electrophoresis indicated at least 95% homogeneity.

TABLE I

MAJOR FRACTIONS FROM *A. nodosum*

Fraction	*Yield*[a] (%)	*Protein* (%)	*Uronic acid* (%)	*Sulfate* (%)	*Fucose* (%)	*Distribution of neutral sugars*[b] (%)				
						Fuc	*Xyl*	*Man*	*Gal*	*Glc*
1	31	3.6	26.4	12.8	15	37	29	21	3	11
2	29	2.4	15.8	20.9	33	73	11	10	2	5
3	18	1.8	6.4	25.2	40	81	9	4	2	4
4	11	8.5	7.1	14.7	19	34	14	15	27	10
5	11	3.1	7.4	8.1	36	71	7	4	14	4

[a] Based on recovered material only. [b] Calculated from gas chromatograms, considering the total area under the five peaks as 100%.

In this way, four homogeneous fractions (Fractions 1–4, Table I) and a fifth fraction consisting of two components in approximately equal amounts (see Figs. 1 and 2) were isolated from *A. nodosum*. Fraction 4 was found to be a galactofucan containing similar proportions of fucose and galactose and a small proportion of uronic acid. Small proportions of galactose are generally associated with fucoidan[3]. Hussein[8] has recently identified from the brown alga *Colpomenia sensuosa* a polymer having a very high galactose content. The presence in *A. nodosum* of a polymer containing about equal amounts of fucose and galactose has not been reported previously. Fractions 1, 2, and 3 (Figs. 1 and 2) comprised ~80% of the purified polymer fractions. Fraction 1 was similar in composition (Table I) and identical in electro-

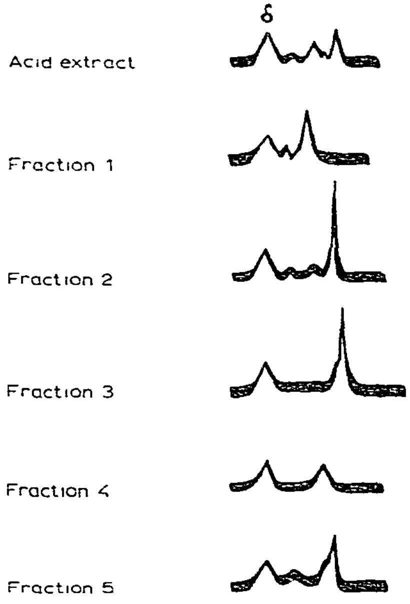
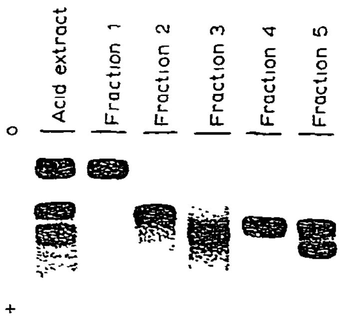


Fig. 1. Ascending pattern at pH 2 of the original extract and purified fractions from *A. nodosum*.



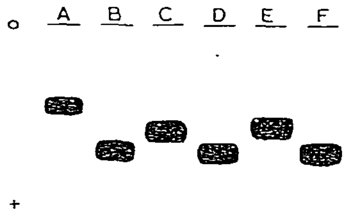
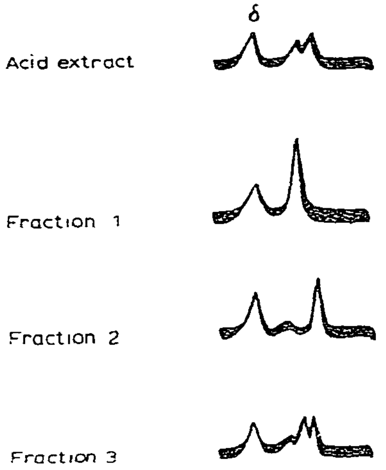


Fig. 2. Cellulose acetate electrophoresis (Toluidine Blue stain) at pH 7.5 of the original extract and purified fractions from *A. nodosum*.

Fig. 3. Cellulose acetate electrophoresis, Toluidine Blue stain, at pH 7.5 of "complex" fractions before, and after, mild acid hydrolysis; A, Fraction 2, A. *nodosum*; B, Fraction 2, *A. nodosum*, hydrolyzed; C, Fraction 3, *A. nodosum*; D, Fraction 3, *A. nodosum*, hydrolyzed; E, Fraction 2, *F. vesiculosus*; F, Fraction 2, *F. vesiculosus*, hydrolyzed.



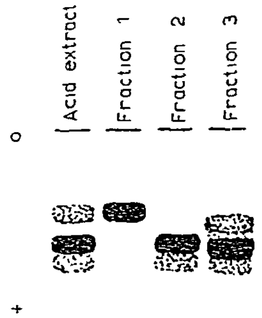


Fig. 4. Ascending pattern at pH 2 of the original extract and purified fractions from *F. vesiculosus*.

Fig. 5. Cellulose acetate electrophoresis (Toluidine Blue stain) at pH 7.5 of the original extract and purified fractions from *F. vesiculosus*.

phoretic mobility to an ascophyllan sample isolated previously[1,2]. Fractions 2 and 3 were very similar in electrophoretic behavior (Figs. 1 and 2) and composition (Table I) and they were difficult to separate. While apparently giving distinct and relatively homogeneous electrophoertic bands, these two fractions are probably subfractions from a group of similar molecules. Mild, acid hydrolysis of both fractions caused the disappearance of the original bands and the appearance of two new bands (Fig. 3), whereas Fractions 1 and 4 remained essentially unchanged. These results suggested that fractions 2 and 3 were "complexes" similar to those previously reported[1,2]. Fraction 5, a relatively minor component, had contents of fucose and uronic acid similar to those of fraction 2, and its individual components behaved as did fractions 2 and 3 on mild, acid hydrolysis. The relatively higher content of galactose and lower electrophoretic mobility of Fraction 5 suggested that it constituted a group of molecules containing some fraction 4 and some less-highly sulfated molecules similar to fractions 2 and 3. No indication of a native, "pure" fucan sulfate could be detected; this observation confirms the earlier work of Larsen, *et. al.*[2].

Results from fractionation of extracts from *Fucus vesiculosus* were generally similar to the foregoing (Table II, Figs. 4 and 5). Fractions 1 and 2 accounted for almost 90% of the isolated material. Fraction 3 was a mixture of at least three components, and the analytical data given in Table II suggest its similarity to Fraction 2.

Fraction 1 from *F. vesiculosus* was similar in composition and electrophoretic mobility to ascophyllan. However, in comparison with fraction 1 from *A. nodosum*, it was slightly higher in content of fucose and lower in that of xylose and uronic acid,

TABLE II

MAJOR FRACTIONS FROM *F. vesiculosus*

Fraction	*Yield*[a] (%)	*Protein* (%)	*Uronic acid* (%)	*Sulfate* (%)	*Fucose* (%)	*Distribution of neutral sugars*[b] (%)				
						Fuc	*Xyl*	*Man*	*Gal*	*Glc*
1	21	6.3	21.9	4.1	18.1	50	15	17	4	14
2	66	5.4	5.6	25.4	48.1	70	7	4	8	11
3	13	3.0	13.9	12.7	40.4	70	7	9	5	9

[a,b]See footnotes [a] and [b], Table I.

and constituted a significantly smaller proportion of the total fucose-containing polysaccharides. Fraction 2 comprised 66% of the material isolated, had a fucose content higher than that of fraction 2 or 3 from *A. nodosun*, and had a high electrophoretic mobility approaching that of a "pure" fucan sulfate. However, hydrolysis with mild acid showed it to be a "complex" of the same general type as fractions 2 and 3 from *Ascophyllum*. Thus, in contrast to the suggestion made by previous workers[2], *Fucus vesiculosus* does not appear to contain a "pure" fucan sulfate.

EXPERIMENTAL

Samples. — Algal samples were collected at Trondheim, Norway (Sept. 13, 1974 for *Ascophyllum nodosum* and Oct. 29, 1974 for *Fucus vesiculosus*) and immediately frozen in acetone cooled with Dry Ice. The frozen samples were ground in a Waring blender, with several changes of Dry Ice-cooled acetone. They were then filtered and air-dried for several days before being milled to a coarse powder in a Wiley mill.

Polymer extraction. — Two preliminary extractions were made on 10-g samples of *Ascophyllum nodosum*. In one, the sample was suspended in 500 ml of water, and the pH was adjusted to 2 with 0.1M hydrochloric acid. The suspension was agitated overnight, filtered, and the filtrate neutralized with M sodium hydroxide. The filter residue was extracted further with 500 ml of water, and the two extracts were combined, dialyzed, and freeze-dried (yield 0.58 g). The second sample was suspended in 500 ml of 0.1M EDTA solution, which had been adjusted to pH 7.5 with M sodium hydroxide, and agitated overnight. The viscous suspension was diluted, filtered, and thoroughly dialyzed. Calcium chloride (M) was slowly added to the solution until no additional precipitate could be filtered off, and the solution was dialyzed and freeze-dried (yield 0.44 g).

For preparative-scale extractions, 40 g of each alga (*A. nodosum* and *F. vesiculosus*) was stirred to a paste with aqueous formaldehyde (2%, 150 ml) and kept overnight in a closed flask. Water (1.5 l) was added, the pH adjusted to 2 with M hydrochloric acid, the suspension stirred for 15 h, and the residue filtered off and reextracted for 5 h with 1.5 liters of water. The combined extracts were neutralized with M sodium hydroxide, dialyzed, and freeze-dried; yield, (from *A. nodosum*) 3.3g; (from *F. vesiculosus*) 2.2 g.

Electrophoresis. — Free-boundary electrophoresis was performed at pH 2 and 7 as described previously[1,9]. Electrophoresis on cellulose acetate was conducted in 0.2M calcium acetate solution (pH 7.5) for 3 h at 0.5 mA/cm, and the strips were stained with Toluidine Blue as described by Seno, *et al.*[10].

Polymer fractionation. — The crude extracts were dissolved in water (1%) and an equal volume of 0.1M aqueous magnesium chloride was added. Fractional precipitation was effected by adding ethanol to a concentration of 50%. The ethanol-soluble and -insoluble fractions were separated by centrifugation, the insoluble one was dissolved in water, and, after removal of ethanol under diminished pressure, both fractions were dialyzed and freeze-dried. In the soluble fraction from both species, the charged polymers were separated from the neutral components by precipitation with cetyltrimethylammonium bromide and subsequent dissolution of the precipitate with 4M sodium chloride solution. The insoluble fraction from *A. nodosum* was separated into its three major components (Fractions 1, 2, 3, Table I) by further fractional precipitation with ethanol of a 1% solution (3 vols.) diluted with 1 vol. of 0.1M aqueous magnesium chloride. Fraction 1 was precipitated in reasonably pure form with 1 volume of ethanol. Fractions 2 and 3 were precipitated at about 2 and 2.5 volumes, respectively. Fractions were reprecipitated until judged by electrophoresis to be sufficiently pure. The soluble fraction from *A. nodosum*, after removal of the neutral glucan, was separated into fractions 4 and 5 (Table I) by dissolving it in water (1%, 4 vols.) and adding 0.1M calcium chloride solution (1 vol.). Ethanol (5 vol.) was added to give a precipitate (Fraction 5, Table I), and a soluble fraction (Fraction 4, Table I). The insoluble fraction from *F. vesiculosus* was resolved into two fractions by dissolving it in water (1%, 1 vol.), diluting with 1 vol. of 0.1M aqueous magnesium chloride, and then adding 0.7 vol. of ethanol. The insoluble fraction (Fraction 1, Table II) and soluble fraction (Fraction 3, Table II) were isolated as already described.

Analytical procedures. — Nitrogen was determined with a Model 1104 Carlo Erba Elemental CNO analyzer. Sulfate[11], uronic acid[12], fucose[13], and the ratio of neutral sugars[14] were determined as described previously.

Mild, acid hydrolysis. — All of the fractions were subjected to hydrolysis with mild acid as described previously by Larsen, *et. al.*[2]. To samples in water (1%, 9 vol.) was added 1 vol. of 0.2M hydrochloric acid and the solution was heated in a water bath for 60 min at 80°.

ACKNOWLEDGMENT

This work was conducted primarily at the Institute of Marine Biochemistry, University of Trondheim while D.G.M. was on sabbatical leave. The assistance and facilities made available by the staff of the Institute are sincerely appreciated. Particular thanks are given to Mrs. Kari Røbech for technical assistance. The authors gratefully acknowledge discussions of this work with Professor Arne Haug (deceased), to whose memory this paper is dedicated. Financial support for D.G.M. was provided, in part, by the Washington Sea Grant Program, as part of the National Sea Grant Program administered by the National Oceanic and Atmospheric Administration of the U.S. Department of Commerce.

REFERENCES

1 B. Larsen, A. Haug, and T. J. Painter, *Acta Chem. Scand.*, 20 (1966) 219–230.
2 B. Larsen, A. Haug, and T. J. Painter, *Acta Chem. Scand.*, 24 (1970) 3339–3352.
3 E. Percival and R. H. McDowell, *Chemistry and Enzymology of Marine Algal Polysaccharides*, Academic Press, London, 1967.
4 A. J. Mian and E. Percival, *Carbohydr. Res.*, 26 (1973) 133–146; 147–161.
5 W. A. Hogsett and R. S. Quatrano, *Plant Physiol.*, 55 (1975) 25–29.
6 A. M. Novotny and M. Forman, *Planta*, 122 (1975) 67–78.
7 R. S. Quatrano and P. T. Stevens, *Plant Physiol.*, 58 (1976) 224–231.
8 M. Magdel-Din Hussein, *Phytochemistry*, 44 (1975) 1866–1868.
9 B. Larsen and A. Haug, *Acta Chem. Scand.*, 17 (1963) 1646–1652.
10 N. Seno, K. Anno, K. Kondo, S. Nagase, and S. Saito, *Anal. Biochem.*, 37 (1970) 197–202.
11 D. G. Medcalf, C. F. Root, C. L. Craney, D. Mukhopadhyay, C. K. Miller, and W. D. Hopewell, *Proc. Int. Seaweed Symp., 7th, University of Tokyo*, 1972, 541–547.
12 C. H. Knutson and A. Jeanes, *Anal. Biochem.*, 24 (1968) 470–480.
13 Z. Dische, *Methods Carbohydr. Chem.*, 1 (1962) 501–505.
14 S. Myklestad, A. Haug, and B. Larsen, *J. Exp. Mar. Biol. Ecol.*, 9 (1972) 137–144.