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STRUCTURAL FEATURES OF A NOVEL GLUCURONOGALACTOFUCAN FROM *Ascophyllum nodosum**

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ABSTRACT

Extraction of the brown alga *Ascophyllum nodosum* with water (adjusted to pH 2) yielded several fucose-containing polymer fractions[1]. One of these fractions was further purified by repeated fractional precipitation with ethanol from aqueous calcium chloride solutions. The resulting, electrophoretically (cellulose acetate) pure polymer was unique in composition, compared with any previously reported polysaccharide from this species. It contained essentially only the neutral sugars fucose and L-galactose (molar ratio 1.1:1), together with glucuronic acid (8%), sulfate (15%), and protein (7%). Major structural features were established by periodate oxidation and methylation analysis. The majority of the fucose residues were sulfated end-units. Glucuronic acid also was found mainly as end-units. The major galactose derivative from the methylated polymer was 3,6-di-*O*-methylgalactose.

INTRODUCTION

The fucose-containing, sulfated polysaccharides from brown algae have been investigated extensively[1–4]. Only two recent studies[5,6] reported polymer fractions high in galactose content in brown algae, and only Percival and Young[5] included any structural data. No galactose-rich fractions have been characterized previously from the widely studied species *Ascophyllum nodosum*[3]. Medcalf and Larsen[1,2] recently re-examined the fucose-containing polymers in this species by using improved purification techniques. A minor fraction (~10% of the total extract) in that study contained approximately equal amounts of fucose and galactose, but was not characterized in detail. The further purification of this fraction and its partial structural characterization are now reported.

RESULTS AND DISCUSSION

The data for the general composition of the purified polymer are shown in Table I. It contained a significant proportion of sulfate, and the nitrogen content

*Dedicated to Dr. Elizabeth Percival.

strongly suggested it was a proteoglycan. All of the uronic acid could be accounted for, after reduction, by the increased glucose content in the neutral sugars. The polymer was composed primarily of fucose and galactose. Treatment of a polymer hydrolyzate with D-galactose oxidase indicated that at least 90% of the galactose was the L form. Other neutral sugars were present in only minor proportions.

TABLE I

GENERAL CHARACTERIZATION OF THE PURIFIED POLYMER FROM *A. nodosum*

		Molar ratio	
$[\alpha]_D^{25}$	−54°	Galactose	1.0
Sulfate (%) (SO_3Na)	15	Fucose	1.1
Uronic Acid (%)	8	Xylose	0.1
Protein (%)	7	Mannose	0.1
		Glucose	trace
		Uronic Acid	0.2
		SO_3Na	0.8

Galactose often has been found as a minor component in brown-algal polymers[3,4]. Only recently has galactose been reported as a major constituent[5,6]. Percival and Young[5] isolated a polymer fraction from *Desmarestia aculeata* that contained galactose, fucose, xylose, and glucuronic acid in the molar ratios 2:1:1.3:1.7. The galactose was the D form, and was found as end-group and (1→3)-linked units.

Major structural features of the glucuronogalactofucan from *A. nodosum* were established by periodate oxidation and methylation analysis. Data for the uptake of periodate and the ratios of neutral sugars in the periodate-oxidized products for both the sulfated and desulfated polymers are shown in Table II. The periodate-labile units in the sulfated polymer were primarily fucose and, considering the methylation data, end-group glucuronic acid. Since end-group fucose and glucuronic acid each take up two mol. of periodate, only ~15% of the original units were periodate-labile. The desulfated polymer consumed over twice as much periodate as the sulfated material. The additional labile units were mainly fucose, as shown by the significant decrease in fucose content of the desulfated and oxidized polymer. This suggested that the sulfate groups were primarily at position 3 of fucose residues. The desulfated polymer still contained some sulfate. Due to the small quantities of material available, accurate analytical results were not obtained. The data available suggested that ~1–2% of sulfate remained. These sulfate residues plus the branched fucose units largely accounted for the fucose remaining after oxidation of the desulfated polymer (Table II). The relative increase in the glucose content of the periodate-oxidized products suggests the presence of periodate-stable glucose residues. While these may be part of the galactofucan polymer, we believe they resulted from a small proportion of the neutral polysaccharide laminarin present as a contaminant.

Analysis (g.l.c.) of the methylated polymer (desulfated and uronic acid reduced), after hydrolysis, produced the data shown in Table III. Only two major peaks were

TABLE II

PERIODATE-OXIDATION DATA

	Original polymer	*Periodate-oxidized polymer*	*Desulfated polymer*	*Periodate-oxidized, desulfated polymer*
Periodate uptake (mol/mol of hexose)	0.31	—	0.68	—
Neutral sugars (molar ratio)				
Galactose	1.0	1.0	—	1.0
Fucose	1.1	0.9	—	0.4
Xylose	0.1	0.1	—	0.2
Mannose	0.1	0.1	—	0.1
Glucose	trace	0.1	—	0.6

TABLE III

METHYLATION ANALYSIS OF THE REDUCED POLYSACCHARIDE

Methylated sugars	*Approximate molar ratio*
Galactose	
3,6-di-*O*-methyl	1.0
3-*O*-methyl	trace
2-*O*-methyl	trace
Fucose	
2,3,4-tri-*O*-methyl	1.1
mono-*O*-methyl	0.1
Glucose	
2,3,4,6-tetra-*O*-methyl	0.1
2,3,6-tri-*O*-methyl	trace

detected. These corresponded to 2,3,4-tri-*O*-methylfucose and 3,6-di-*O*-methylgalactose. Much smaller amounts of 2,3,4,6-tetra-*O*-methylglucose and mono-*O*-methylfucose were also found.

These data on periodate oxidation and methylation suggested that the polymer consisted largely of a chain of L-galactosyl residues to which were attached single L-fucosyl branches. Glucuronic acid was also present as end-units. A few of the fucose branches had additional units attached to two of their available hydroxyl groups. About two-thirds of the end-units contained a sulfate group at position 3. From the data available, the galactose chain may be either (1→4)-linked with branches at position 2, or (1→2)-linked with branches at position 4. Literature precedence[3] suggests that the former is more likely.

EXPERIMENTAL

The algal sample, polymer extraction, and polymer fractionation procedures have been described[1]. The purity of the various fractions during fractional precipitation was followed by cellulose acetate electrophoresis[1]. Fraction 4 (100 mg) from the previous study[1] was redissolved in water (1%, 1 vol.), and the solution diluted with an equal volume of 0.01M calcium chloride. Ethanol was added slowly until a definite precipitate was detected. After isolation of the precipitate by centrifugation, ethanol was again added to the supernatant solution until a precipitate formed. This process was repeated until no further precipitation occurred when an additional volume of ethanol was added. All precipitates were dissolved in water, and the solutions dialyzed and freeze-dried. Fractions were analyzed by electrophoresis, and those containing significant amounts of the major component were combined and refractionated as above. This procedure was repeated until ~30 mg of material showing a single band on cellulose acetate electrophoresis was obtained. The neutral sugar composition before and after further fractionation of a portion of this material was unchanged.

General procedures. — Nitrogen, sulfate, uronic acid, fucose, and the ratio of neutral sugars were determined as described previously[1]. Desulfation of a portion of the polymer was effected with 0.3% methanolic hydrogen chloride[7].

Uronic acid identification. — The sample (5 mg) was reduced by the procedure of Taylor and Conrad[8]. Neutral sugars were analyzed before and after reduction.

Identification of L-*galactose.* — A portion of the hydrolyzed polymer was analyzed for D-galactose by using the Worthington Galactostat procedure (Worthington Biochemical Corporation, Freehold, N.J., U.S.A.).

Periodate oxidation. — Periodate oxidation was performed on the sulfated and desulfated polymer by using the micro-procedure of Avigad[9]. After oxidation was complete, the excess of periodate was reduced with ethylene glycol, and the solution dialyzed and freeze-dried. The resulting polymers were subjected to neutral-sugar analysis.

Methylation analysis. — The polymer (desulfated and uronic acid reduced) was methylated by the micro sodium hydride–methyl sulfoxide procedure[10]. Complete methylation was indicated by the absence of i.r. absorption for hydroxyl in the methylated product. The methylated polymer was hydrolyzed by using the 72%–8% sulfuric acid method[11]. The methylated sugars were reduced and acetylated[12], and the resulting alditol acetates analyzed by g.l.c. Identification was based on relative retention times under three different conditions, with the alditol acetates of 2,3,4,6-tetra-*O*-methyl-D-glucose and 3-*O*-methyl-D-xylose as standards. Conditions used were isothermal ECNSS-M[13], temperature-programmed ECNSS-M[14], and isothermal OV-225[15].

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