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STRUCTURAL FEATURES OF A SULPHATED, FUCOSE-CONTAINING POLYSACCHARIDE FROM THE BROWN SEAWEED *Dictyota dichotoma*

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ABSTRACT

A sulphated heteropolysaccharide (~15% of the acid-extractable material) isolated from the brown alga *Dictyota dichotoma* contains residues of D-glucuronic acid, D-galactose, D-mannose, D-xylose, and L-fucose[1]. Partial hydrolysis of the polysaccharide with acid gave one neutral and two acidic oligosaccharides. The behaviour towards periodate of the polysaccharide before and after partial hydrolysis, alkali-treatment, and methanolysis has been studied. Evidence is thereby provided that the polysaccharide is partially sulphated and composed of (1→4)-linked residues of D-glucuronic acid, D-galactose, D-mannose, and D-xylose, and (1→2)-linked L-fucose.

INTRODUCTION

We have described elsewhere[1] the isolation of a polysaccharide (2.4% of the alga) of an acid-soluble extract of the alga *Dictyota dichotoma*. The polysaccharide contains[1] mainly glucuronic acid, galactose, and fucose with smaller amounts of D-mannose and xylose, and is devoid of protein. By analogy with similar polysaccharides of brown algae, it is assumed that, apart from L-fucose, the rest of the sugars are D isomers. The polysaccharide has high anticoagulating activity. We now report on the structural features of this polysaccharide.

EXPERIMENTAL

General. — Paper chromatography (p.c.) was performed on Whatman No. 1 paper with *A*, 1-butanol–ethanol–water (40:11:19); *B*, 1-butanol–pyridine–water (3:1.1:1.5); and *C*, ethyl acetate–acetic acid–water[2] (3:1:3). Detection was effected with aniline hydrogen phthalate, alkaline silver nitrate, and aniline–xylose reagents[3]. Paper electrophoresis was performed on an Elphor apparatus with a pyridine–acetic acid buffer[4] (0.05M) at pH 6.0. A potential of 300 volts, giving a current of 0.2mA/strip was applied for 2.5 h at room temperature. The degree of polymerisation (d.p.) of oligomers was determined by the method of Timell[5].

The brown alga *Dictyota dichotoma* was collected in August 1972 from Roushdy

at Alexandria. The alga was washed with water to remove foreign substances, and then air-dried and milled.

Preparation of the purified, sulphated heteropolysaccharide. — The partially purified polysaccharide (*P*) isolated[1] in 6% yield from *D. dichotoma* had total carbohydrate, 69.5; ash, 14.0; SO_4^{2-}, 16.0%. Complete hydrolysis of the polysaccharide with acid afforded (p.c., solvent *A*) glucuronic acid, galactose, glucose, mannose, xylose, and fucose.

From a solution of *P* (3 g), polysaccharide *R* (1.2 g) was recovered as described before[1]. It had $[\alpha]_D^{25}$ −79.3° (*c* 0.5, water) (Found: SO_4^{2-} 16.6%). Complete hydrolysis of *R* with acid afforded glucuronic acid, galactose, mannose, xylose, and fucose in the molar ratios 2.3:2.6:1.0:2.0:2.9 (p.c., solvent *A*).

Partial, acid hydrolysis of the purified polysaccharide. — Polysaccharide *R* (0.3 g) was hydrolysed in 0.5M oxalic acid (10 ml) at 100° for 5 h. Thereafter, the hydrolysis mixture was cooled and dialysed against distilled water (4 × 100 ml), and the combined dialysates were neutralized with calcium carbonate, filtered, treated with Lewatit S-100(H^+) resin, and concentrated to 15 ml (product *C*). The non-dialysable material was centrifuged, the supernatant solution was freeze-dried (product *D*, 0.04 g), and the sediment was dried under reduced pressure at 40° to afford product *E* (0.04 g).

Product *C* was transferred to a column (2 × 9 cm) of Amberlite IR-400(AcO^-) resin, which was washed with water until the effluent gave a negative phenol–sulphuric acid test. This procedure yielded a neutral fraction that gave one neutral oligosaccharide (**1**) by preparative p.c. (solvent *A*). Further elution with 0.5M sulphuric acid (1 litre) furnished an acidic fragment **2** that migrated as a single band on electrophoresis, but yielded two acidic oligosaccharides ($\mathbf{2}_1$ and $\mathbf{2}_2$) in p.c. (solvent *A*).

Reduction and hydrolysis of the oligosaccharides. — Following the procedure of Perila and Bishop[6], samples (1–2 mg) of the oligosaccharides were dissolved in water (0.5 ml) and treated with sodium borohydride (0.05 g) in water (1 ml) at room temperature for 18 h. The solutions were neutralized with acetic acid, treated with Lewatit S-100(H^+) resin, and evaporated, and methanol was then thrice distilled from the residue. The resulting, reduced oligosaccharides were hydrolysed with 0.3M hydrochloric acid (2 ml) at 97° for 4 h, and the products were subjected to quantitative p.c. (solvent *A*).

Desulphation of the polysaccharide. — Polysaccharide *R* (0.3 g) was treated with sodium hydroxide according to the method of Rees[7]. Thereafter, the alkaline solution was neutralized with Lewatit S-100(H^+) resin, dialysed for several hours against distilled water, and then freeze-dried to give *H* (0.18 g) (Found: SO_4^{2-}, 11.25%). Complete hydrolysis of *H* with acid gave (p.c.) glucuronic acid, galactose, mannose, xylose, and fucose in the molar ratios 2.6:2.5:1.00:0.90:2.48.

Polysaccharide *R* (0.3 g) was shaken with 0.1M methanolic hydrogen chloride[8] (50 ml) at room temperature for 26 h. The residual material was dissolved in water, dialysed against distilled water, and then freeze-dried to give *I* (0.21 g) (Found: SO_4^{2-}, 13.32%). Complete hydrolysis of *I* with acid gave (p.c., solvent *A*) glucuronic

acid, galactose, mannose, xylose, and fucose in the molar ratios 1.9:3.3:1.0:1.2:2.8.

Periodate oxidation of polysaccharides R, D, H, and I. — These polysaccharides were separately oxidised with 16mM sodium metaperiodate (250 ml) at 2° in the dark[8]. Aliquots (5 ml) were withdrawn at intervals and used for the determination of consumed periodate[9] and released formic acid[10]. At the end of the oxidation process, the released formaldehyde was determined[11].

Reduction and hydrolysis of the periodate-oxidised materials. — Reduction was performed as reported by Abdel-Fattah *et al.*[12], and hydrolysis of the polyalcohols was effected by the method of Drummond *et al.*[13]. Each hydrolysate was treated with Lewatit S-100(H^+) resin and subjected to p.c. (solvents *A*, *B*, and *C*), using the appropriate reference compounds.

RESULTS AND DISCUSSION

Partial hydrolysis of the purified polysaccharide *R* with oxalic acid afforded dialysable and non-dialysable materials. The dialysable material *C* (66% of *R*) contained glucuronic acid, galactose, mannose, xylose, and fucose in the molar ratios 1.45:6.07:1.00:3.14:9.29, together with a neutral oligosaccharide (**1**) and two acidic oligosaccharides ($\mathbf{2_1}$) and ($\mathbf{2_2}$).

The neutral oligosaccharide **1** (d.p. 9) contains mannose and xylose in the molar ratio 2:1. The acidic oligosaccharide $\mathbf{2_1}$ (d.p. 5; 8 SO_4^{2-} groups) contains mannose and xylose in the molar ratio 4:1, and acidic oligosaccharide $\mathbf{2_2}$ (d.p. 3; 3 SO_4^{2-} groups) contains glucuronic acid, mannose, and xylose in the molar ratios 1:1:1. Reduction of the oligosaccharides with borohydride, followed by hydrolysis and p.c. (solvent *A*), gave xylitol, indicating that xylose is the reducing terminus of each oligosaccharide.

The presence of only mannose and xylose in the neutral oligosaccharide **1** indicates that some of these sugar residues in the polysaccharide carry no sulphate groups. The presence of sulphate in both acidic oligosaccharides ($\mathbf{2_1}$ and $\mathbf{2_2}$) indicates that other residues of mannose and/or xylose carry sulphate groups. The appearance of mannose and xylose in the isolated oligosaccharides suggests the presence in the polysaccharide of branches constituted of at least neutral and partially sulphated mannose and xylose residues. No oligosaccharide containing either fucose or galactose was isolated. On the other hand, the presence of only one molecule of glucuronic acid in the acidic oligosaccharide $\mathbf{2_2}$, together with its low proportion as monomer in product *C*, indicated a certain resistance of these sugar residues to partial hydrolysis.

The non-dialysable material *D* (13% of *R*) contained 15% of sulphate and on hydrolysis gave glucuronic acid, galactose, mannose, xylose, and fucose in the molar ratios 2.9:2.1:1.4:1.0:2.0 Product *E* (13% of *R*) contained 8.46% of sulphate and gave only galactose on hydrolysis.

Thus, the inner part of the polysaccharide seems to be similar to its branches in being partially sulphated and composed of glucuronic acid, galactose, mannose, xylose, and fucose.

Partial desulphation of the polysaccharide with alkali (product *H*) or by methanolysis (product *I*) led to some loss of material and to partial removal of sulphate. Product *H* gave a positive resorcinol test[14] for 3,6-anhydrohexose, indicating the presence of 3- and/or 6-sulphate in the polysaccharide.

Oxidation of polysaccharide *R* with periodate stopped after 24 h and the reduction of periodate was then 0.53 mol per sugar residue. The low reduction of periodate is partly due to sulphated residues and may also indicate the presence of branch points and/or 1,3-linked units.

From the amount of formaldehyde (0.021 mol/residue) and formic acid (0.10 mol/residue) released during the oxidation of the polysaccharide, average degrees of polymerisation (d.p.) of 47.6 and 10.0 were calculated for the whole polysaccharide molecule and each of its branches, respectively. On the basis of the d.p. of the polysaccharide and its monosaccharide composition, it may be deduced that the whole polysaccharide molecule is composed of ~10 glucuronic acid, 11 galactose, 4 mannose, 9 xylose, and 13 fucose residues. Hydrolysis of the derived polyalcohol gave (p.c.) erythronic acid, glyceric acid, glycerol, propane-1,2-diol, threitol, erythritol, glycolaldehyde, and detectable amounts of the sugars constituting the parent polysaccharide.

The formation of erythronic acid demonstrated the presence of (1→4)-linked glucuronic acid residues, while the detection of glyceric acid indicated the presence of glucuronic acid as the non-reducing end of the polysaccharide backbone. Glycerol would be derived from (1→4)-linked xylose residues, propane-1,2-diol from (1→2)-linked fucose residues, threitol from (1→4)-linked galactose residues, and erythritol from (1→4)-linked mannose residues. The formation of glycolaldehyde provided strong evidence for the presence of (1→4)-linked glucuronic acid, galactose, and mannose residues.

The resistance of some of the sugar residues might be due to the presence of sulphate and branch points. The insignificant decrease in the fucose content of the polysaccharide by treatment with alkali (product *H*) demonstrates the resistance of its sulphate to alkali treatment and hence its presence on C-4 or C-3 of 1,2-linked fucose (alkali-lability of sulphate groups necessitates their presence on vicinal, *trans*-hydroxyl groups[15]).

The non-dialysable material *D* (13% of *R*; 15% of SO_4^{2-}), obtained by partial hydrolysis of the polysaccharide *R*, reduced 1.1 mol of periodate per sugar residue. The amount of formaldehyde and formic acid released (0.060 and 0.625 mol/sugar residue, respectively) was consistent with average d.p. values of 16.7 and 1.6 for *D* and each degraded branch. The latter value, compared with that of the whole polysaccharide molecule, indicates that most of the branch residues were removed. From the monosaccharide content of product *D*, it is concluded that there are ~6 glucuronic acid, 4 galactose, 3 mannose, 2 xylose, and 4 fucose residues in the polysaccharide backbone.

The hydrolysate of the polyalcohol derived from *D* contained traces of intact sugar residues and a higher proportion of erythronic acid than for the parent poly-

saccharide. This result indicates that, by partial hydrolysis of the polysaccharide, more glucuronic acid residues in the backbone became susceptible to oxidation by periodate.

The alkali-treated polysaccharide *H*, recovered in 60% yield, consumed 0.73 mol of periodate per sugar residue, indicating that this product was more susceptible towards periodate than the parent polysaccharide. From the amount of formaldehyde (0.031 mol/residue) and formic acid (0.139 mol/residue) released during periodate oxidation, average d.p. values of *H* and each branch were 32 and 7.2, respectively. The hydrolysate of the polyalcohol derived from *H* contained high proportions of galactose and fucose.

Partial desulphation of the polysaccharide by methanolysis gave product *I* (recovered in 70% yield), which consumed 0.47 mol of periodate per sugar residue. The small decrease of susceptibility of *I* towards periodate oxidation might be due to the removal, during methanolysis, of some sugar residues that were originally vulnerable to attack by periodate. The amount of formaldehyde and formic acid released (0.034 and 0.12 mol/residue, respectively) was consistent with average d.p. values of 29.4 and 8.3 for *I* and each branch, respectively. The hydrolysate of the polyalcohol derived from *I* contained similar proportions of intact sugars and products found in the polyalcohol hydrolysate of the parent polysaccharide. This indicated that the methanolysis conditions were insufficient to desulphate most of the sulphated sugar residues in the polysaccharide molecule.

The results of the present work show that polysaccharide *R* is made up of highly branched molecules containing the following structural features (1→4)-linked D-glucuronic acid, (1→4)-linked D-galactose, (1→2)-linked L-fucose, (1→4)-linked D-mannose, and (1→4)-linked D-xylose residues. Many of these residues are sulphated.

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