Acta Chemica Scandinavica B 29 (1975) 337—340

Structural Studies of the Extracellular Polysaccharide Produced by the Diatom *Chaetoceros curvisetus* Cleve

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Methylation studies and partial acid hydrolysis have been carried out on the extracellular polysaccharide produced by the diatom *Chaetoceros curvisetus*. The polysaccharide contains the sugars galactose, rhamnose, and fucose and is partially sulfated. The presence of fucose in both furanose and pyranose forms within one molecule is reported for the first time. The fucofuranose is present as end groups, 1,2-linked and as branch points. Fucopyranose is mainly present as branch points in the polysaccharide, some being 1,3-linked, and a small fraction as end groups. The main part of rhamnose is 1,2-linked and galactose is mainly 1,3-linked.

Several diatoms are known to produce extracellular polysaccharide.[1] The production of extracellular polysaccharides produced by species of the *Chaetoceros* family, has been studied [2,3] and three members of this family were shown to excrete significant amounts of polysaccharide into the surrounding medium when being in the stationary growth phase. The three species were *C. affinis* (clone CH1), *C. curvisetus* (CH24), and *C. decipiens* (CH40).

These polysaccharides were all sulfated and contained the sugars galactose, rhamnose, and fucose in different proportions. Structural studies on the polysaccharide produced by *C. affinis* [4] showed that this polysaccharide was highly branched and had a complex structure. Methylation studies showed that the polysaccharide produced by *C. decipiens* had a very similar structure,[5] while the polysaccharide produced by *C. curvisetus* was different. The present paper describes structural studies of this polysaccharide.

RESULTS AND DISCUSSION

The cultivation of the diatom *C. curvisetus* and isolation of the extracellular polysaccharide was performed as for *C. affinis*.[2,6]

The polysaccharide (CH24) had $[\alpha]_D = -68°$, a carbohydrate content of 80 %, $-SO_3Na$ was 6.9 % determined by the method of Antonopoulos.[7] Potentiometric titration gave an equivalent weight of 950, which, if the only anion present is sulfate, was calculated to correspond to approximately 10 % $-SO_3Na$.

Subjected to free boundary electrophoresis at pH 2 and pH 7, the polysaccharide moved as a single, anionic compound, with approximately the same mobility at both pH values, also indicating that the anion is sulfate (or another strong acid).

Complete acid hydrolysis of the polysaccharide and analysis by GLC of the derived alditol acetates [6,12] showed the presence of rhamnose, fucose, and galactose in the proportions 0.3 to 3.5 to 1.0. No other sugars were detected. The configuration of the sugars has not been determined due to shortage of material.

Preliminary methylation experiments, combined with analysis of the derived partially methylated alditol acetates by GLC-MS,[4] indicated that some of the fucose present as end groups was in the furanose form. It is well known that end groups in furanose form are readily released from the rest of the polysaccharide under weak acidic conditions. In the extracellular polysaccharide produced by *C. affinis* (CH1) rhamnopyranose is responsible for the main part of the non reducing end groups.[4] CH1 and CH24 polysaccharides were

Table 1. Composition of polysaccharide before and after partial hydrolysis.

	Carbohydrate content (mg)	Sugar ratio Rhamnose	Fucose	Galactose
CH24	19	0.3	3.5	1.0
1 h	10	0.3	2.3	1.0
3 h	7.5	0.2	1.6	1.0

both subjected to weak acid hydrolysis (see Experimental). The hydrolysates of both polysaccharides were dialysed, and the dialysates tested for carbohydrate.[8] The dialysate from CH1 polysaccharide contained no carbohydrate, while that from CH24 polysaccharide contained considerable amounts. When analysed by TLC, and GLC after conversion to alditol acetates, no other monosaccharide than fucose was found in the dialysate.

The starting material and the products after 1 and 3 h of hydrolysis, were all analysed for sugar ratios by GLC and for carbohydrate contents by the phenol-sulfuric acid method,[8] using as standards solutions containing the sugars in appropriate ratios (Table 1). It can clearly be seen from Table 1 that fucose is lost during this hydrolysis, and the fact that it is readily released indicates that it is present in the furanose form.

The three polysaccharide samples were subjected to methylation,[9,10] hydrolysed and converted into the partially methylated alditol acetates. These were all analysed by combined GLC-MS (col. OV-225).[4] The identifications were thus confirmed both by their retention times (T) and mass-spectra (Table 2). The compounds with T-values 0.56, 0.96 and 1.33, gave a MS-fragmentation pattern different from those derived from partially methylated alditol acetates originally present in pyranose form. The compound with T = 0.56 gave as the main fragments *m/e* 59, 117, and 175 (Fig. 1), which previously has been shown to derive from the alditol acetate corresponding to 2,3,5-tri-*O*-methylfucose.[11] That with T = 0.96 gave as main fragments *m/e* 59, 175, and 189 (Fig. 1). These can only be obtained from the alditol acetate corresponding to 3,5-di-*O*-methylfucose. The compound with T = 1.33 gave as the main fragment *m/e* = 59 (Fig. 1), indicating that the compound is the alditol acetate corresponding to 5-*O*-methylfucose.

The presence of these three methylated products shows that fucose in the furanose form is not only present as end groups, but also as part of the chain and as branch points. The presence of these forms in a polysaccharide

Table 2. GLC analysis of the methylated alditol acetates.

Retention times [a] (T)	Column OV – 225	Weights calculated from GLC (mg) Start	1 h	3 h
2,3,4-Tri-*O*-methylrhamnose	0.47	0.2	0.2	0.5
2,3,5-Tri-*O*-methylfucose	0.56	2.9	1.2	0.6
2,3,4-Tri-*O*-methylfucose	0.60	tr	0.2	0.1
3,4-Di-*O*-methylrhamnose	0.88	1.0	0.8	0.5
3,5-Di-*O*-methylfucose	0.96	4.0	1.6	1.0
2,4-Di-*O*-methylfucose	1.04	1.8	1.3	0.8
2,3,4,6-Tetra-*O*-methylgalactose	1.15	0.3	0.8	0.5
5-*O*-Methylfucose	1.33	1.8	0.3	0.2
2-*O*-Methylfucose	1.41	1.1	0.2	0.2
4-*O*-Methylfucose	1.71	2.9	1.3	1.1
2,4,6-Tri-*O*-methylgalactose	1.92	1.5	1.5	1.5
4,6-Di-*O*-methylgalactose	2.95	1.3	0.5	0.5

[a] Relative to 2,3,4,6-tetra-*O*-methyl-D-glucitol 1,5-diacetate.

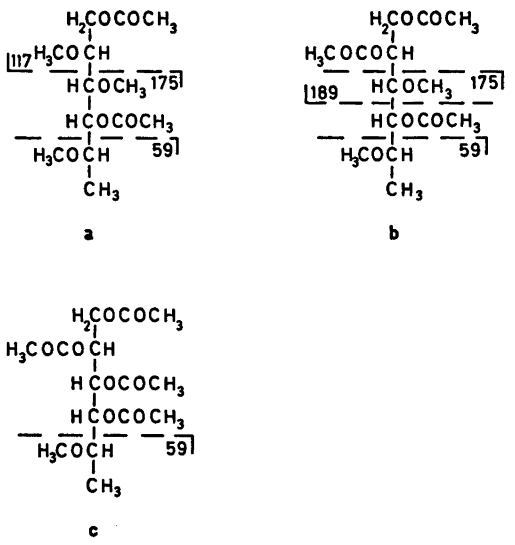

Fig. 1. Alditol acetates corresponding to partially methylated fucose. Primary fragments obtained by mass-spectrometry. a. 2,3,5-tri-*O*-methylfucose; b. 3,5-di-*O*-methylfucose; c. 5-*O*-methylfucose.

has not been reported previously.

Table 2 also shows the presence of fucose in pyranose forms, both as end groups, within the chain, and as branch points, the main part as the latter. The rhamnose is mainly present as 1,2-linked pyranose units, while the galactose is mainly found as 1,3-linked; a smaller part as branch points, then being 1,3- and 1,2-linked, both in pyranose forms. The mass spectra of these compounds corresponded to those published by Björndal *et al.*[11] When subjected to weak acid hydrolysis, only fucose was lost (Table 1). The approximate weights of the alditol acetates obtained from the methylated original polysaccharide and the methylated residual polysaccharides after 1 and 3 h of hydrolyses were estimated from the GLC result by determining the area under the peaks. This will not give a correct estimate of the amounts of methylated products obtained from the polysaccharides, but may be used for comparing the composition of the three samples.

The major loss of fucose is from the part present as furanose. Fucopyranose is also lost; the main change occurring in the fucopyranose present as branch points. This shows that some of the fucose residues lost were linked to fucopyranose residues being branch points; the main part has probably been linked to fucose in the 2-position, as the weight of 4-*O*-methylfucose has dropped most. 2-*O*-Methylfucose can be derived from fucose present both in the pyranose and furanose form. There is a considerable loss of 2-*O*-methylfucose during the first period of the hydrolysis (Table 2). This indicates that some of the fucose present as 2-*O*-methylfucose in the methylated starting material is present as furanose in the polysaccharide.

The ratio between rhamnose present as end groups to that present as part of the chain increases from 0.2:1 to 1:1 as hydrolysis time increases. From this it can be deduced that some of the fucose lost was linked to rhamnose at C-2, which then gives rise to end groups when the fucose residues disappear. The changes in the amounts of the different methylated galactose derivatives are relatively small. The main being in 4,6-di-*O*-methylgalactose, indicating that some half-estersulfate and/or fucose attached to galactose is released during hydrolysis.

The results above show that the extracellular polysaccharide produced by the diatom *C. curvisetus* is a highly branched polysaccharide of a complex structure. Fucose is present both in furanose and pyranose forms, the former being responsible for the main part of the end groups. Fucofuranose is also present as part of chains and as branch points. Fucopyranose appears to be responsible for the main part of the branch points of the polysaccharide. Some of the fucose appears to be linked to rhamnose at C-2. Rhamnose and galactose are responsible for a small part of the end groups. Galactose is mainly present at the inner part of the molecule.

EXPERIMENTAL

Weak acid hydrolysis. The polysaccharide was dissolved (1 %) in 0.01 N sulfuric acid. The mixture was heated at 100 °C and aliquots withdrawn after 1 and 3 h. The hydrolysates were, after cooling, neutralized with sodium hydrogencarbonate. The hydrolysates were freeze dried after dialysis.

Carbohydrate contents and sugar ratios were measured after all the above-mentioned steps. Other experimental details were as previously described.[4]

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Received September 21, 1974.