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Note

Structural studies of the reserve glucan produced by the marine diatom *Skeletonema costatum* (grev.) Cleve

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Structural investigations of a water-soluble glucan from fresh-water diatoms and from the marine diatom *Phaeodactylum tricornutum*, respectively, have shown[1,2] that they are (1 → 3)-β-D-linked with some branching at position 6. Polysaccharides of this type are called chrysolaminarans. The main difference between laminaran and chrysolaminaran is that the latter appears to be devoid of chains terminating in mannitol[1,2].

Extraction of the freeze-dried alga *Skeletonema costatum* with aqueous acid gave a solution from which the polysaccharide was recovered in 32% yield. The polysaccharide had $[\alpha]_D^{20}$ −7° (*c* 0.2, water), and a carbohydrate content of 98%. Methanolysis followed by conversion of the products into the corresponding trimethylsilyl ethers revealed glucose to be the only component. Also, p.c. of an acid hydrolysate revealed only D-glucose.

The molecular weight (M_w) determined by gel filtration, with reference to dextran, was ~6,200, and osmometry gave a molecular weight (M_n) of 13,000. The latter method is the more reliable, since gel filtration probably gives too low a value due to differences in shape of the polysaccharide and the reference dextran.

The polysaccharide was subjected to methylation analysis, and the resulting methylated alditol acetates were analysed by g.l.c.–m.s.[5]. The results (Table I) show that the polysaccharide is a (1 → 3)-linked glucan with some branching at positions 6 and 2. Branching at the latter position has not been observed previously in glucans from other diatoms[1,2] or benthic algae[1,2]. The peak areas allow a semiquantitative estimation of the relative amounts of the methylated derivatives. The ratio of end groups to branch points is acceptable; the later peaks generally have larger areas. The methylation studies indicate an average chain-length of ~11.

The polysaccharide consumed 0.23 mol. of periodate per sugar unit, corresponding to ~9 residues per chain. Periodate oxidation of a non-reducing end-group

TABLE I

G.L.C. DATA FOR THE METHYLATED ALDITOL ACETATES

Alditol acetate derived from	T[a]	*Relative peak area*
2,3,4,6-Tetra-*O*-methylglucose	1.00	1.0
2,4,6-Tri-*O*-methylglucose	1.70	8.9
4,6-Di-*O*-methylglucose	3.05	0.8
2,4-Di-*O*-methylglucose	3.64	0.7

[a]Retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

yields glycerol after reduction of the polyaldehyde followed by hydrolysis; (1 → 3)-linked glucose residues remain uncleaved. The ratio of glycerol to glucose was 1:10.8, giving a chain length of ~11, which is consistent with the result obtained by the methylation studies.

The results obtained with the reserve polysaccharide isolated from the diatom *S. costatum* show that the polysaccharide is a (1 → 3)-linked β-D-glucan with branches at positions 2 and 6. The latter branching-location has previously been found in glucans from other algae, *e.g.*, in laminaran, para-amylon, and chrysolaminaran[1,2]. Branching at position 2 has not been found previously in algal reserve polysaccharides.

EXPERIMENTAL

The cultivation of *S. costatum* was carried out as described previously[3], with a culture volume of 45 l. The cells were collected by centrifugation in a simple, semi-continuous centrifuge[4]. For preparation of the glucan, 300 mg of freeze-dried algae was extracted with 20 ml of 0.05M sulphuric acid for 1 h at 4°. After filtration, the extraction was repeated with more (10 ml) 0.05M acid. The two filtrates were combined, made neutral with 0.1M sodium hydroxide, and dialyzed (4×) against a large volume of distilled water. The non-dialyzable fraction was concentrated, filtered, and freeze-dried. The product (~95 mg) was stored at 4°.

Concentrations were performed under diminished pressure at 40°. Dialysis was carried out against distilled water. The $[\alpha]_D$ value was measured at 20° with a Perkin–Elmer 141 polarimeter. Carbohydrate content was determined by the phenol–sulphuric acid[7] method using D-glucose as the standard. The molecular weight by gel-filtration was determined on a column (1 × 25 cm) of Sephadex G-100 calibrated against dextrans of known molecular weights. Fractions (5 drops) were collected, using Tris–HCl buffer (0.05M, pH 7.3) as eluant. The molecular weight was also determined by osmometry at 20° with a Knauer membrane osmometer.

Hydrolysis was carried out in 90% formic acid[6] for 6 h at 100°. The mixture was then diluted with 5 vol. of water and heating was continued for 2 h. P.c. was

carried out on Whatman No. 1 paper with ethyl acetate–pyridine–water (8:2:1), and detection with Glucostat^{R} (Worthington Biochemical Corporation).

G.l.c. was performed on a Varian 1400 gas chromatograph fitted with a flame-ionization detector and glass columns (360 × 3 mm) of (*a*) 3% of SE-52 on Varaport 30 at 120° for 8 min, followed by an increase of 4°/min, and (*b*) 3% of OV-225 on Varaport 30 at 200°.

G.l.c.–m.s. was performed with column (*b*) fitted in a Varian 1400 gas chromatograph coupled to a Varian CH-7 low-resolution mass spectrometer. The instrumental details are published elsewhere[8].

Methanolysis was performed by heating the sample (2.80 mg) with M hydrogen chloride in methanol (0.5 ml) at 85° for 20 h, and then preparing[9] the product for g.l.c. as the *O*-trimethylsilyl derivatives.

Methylation was performed by the modified Hakomori method[5], using 5 mg of polysaccharide. The methylated product was hydrolysed, and the partly methylated monosaccharides were converted[10] into the corresponding alditol acetates for analysis by g.l.c. and g.l.c.–m.s.

Periodate oxidation was carried out as described by Ferrier and Aspinall[11], using 9.1 mg of polysaccharide in 15mM $NaIO_4$ (5 ml). Samples (100 µl) were removed at intervals for measurement of periodate consumption. The reaction was terminated after 8 h by adding ethylene glycol (1 ml). After dialysis, the polyaldehyde was reduced with sodium borohydride (10 mg) and then methanolysed, and the products were converted into the corresponding *O*-trimethylsilyl derivatives prior to analysis by g.l.c. on column (*a*).

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REFERENCES

1 A. Beattie, E. L. Hirst, and E. Percival, *Biochem. J.*, 79 (1961) 531–537.
2 C. W. Ford and E. Percival, *J. Chem. Soc.*, (1965) 7035–7041.
3 S. Myklestad, *J. Exp. Mar. Biol. Ecol.*, 15 (1974) 261–274.
4 T. Lien and G. Knudsen, *Lab. Pract.*, (1975) 29.
5 B. Smestad, A. Haug, and S. Myklestad, *Acta Chem. Scand., Ser. B*, 28 (1974) 662–666.
6 E. Percival, *Carbohydr. Res.*, 7 (1968) 272–283.
7 M. Dubois, K. A. Gilles, J. K. Hamilton, O. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
8 E. Jellum, D. Stokke, and L. Eldjarn, *Clin. Chem.*, 18 (1972) 800–809.
9 V. N. Reinhold, *Methods Enzymol.*, 25 (1972) 244–249.
10 H. Björndal, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 21 (1967) 1801–1804.
11 G. O. Aspinall and R. J. Ferrier, *Chem. Ind. (London)*, (1957) 1216–1217.
12 E. Percival, in W. Pigman and D. Horton (Eds.), *The Carbohydrates*, Vol. IIB, Academic Press, New York, 2nd edition, 1970, pp. 541–544.