Carbohydrate Research, 153 (1986) 330–333
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Structural studies of chrysolaminaran from the ice diatom *Stauroneis amphioxys* (Gregory)

MALCOLM J. MCCONVILLE, ANTONY BACIC, AND ADRIENNE E. CLARKE
Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville, Victoria 3052 (Australia)

(Received August 26th, 1985; accepted for publication in revised form, November 22nd, 1985)

The main reserve material of diatoms is the water-soluble β-D-glucan, chrysolaminaran. The major linkage in chrysolaminaran extracted from different diatom species is (1→3)-β-glucosidic, but there may be variation in the position and type of other linkages, as well as variation in the molecular weight[1–3]. We report here a study of the structure of chrysolaminaran isolated from axenic cultures of the ice diatom *Stauroneis amphioxys* and from a diatom-dominated algal community collected from Antarctic sea ice.

Hot-water extraction of *S. amphioxys* cells removed 64–70% of the cellular carbohydrate, the remainder being cell-wall residue as judged by microscopy. Size fractionation of the extract by ultrafiltration gave two groups of polysaccharides: a low-molecular-weight fraction (between 1,000–10,000) shown (after hydrolysis and conversion of products into their corresponding alditol acetates) to contain only glucose, and a high-molecular-weight fraction (>10,000) that had a highly complex monosaccharide composition with glucose as a minor constituent (<10%). The low-molecular-weight glucan accounted for 70–80% of the extracted carbohydrate.

The molecular weight of the glucan was determined by l.c. with an Ultrapac TSK G2000 gel-permeation column, calibrated with polyethylene glycol standards. This gave an average molecular weight of 4,000, corresponding to a d.p. of ~24.

Methylation analysis showed that the glucan consisted mainly of 3-linked glucosyl residues with some branching through O-2 and O-6 (Table I). On average, there was slightly less than one of each of these branch points per molecule. The ratio of terminal to interchain residues indicates an average chain-length of 19. In addition, small proportions of 1,3,4,5-tetra-*O*-acetyl-2,6-di-*O*-methylglucitol were found consistently in several analyses of *S. amphioxys* chrysolaminaran, indicating a very low level of branching through O-4. Low levels of 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucitol detected in these analyses are also evident in the methylation analyses of other chrysolaminaran[1] and laminaran[1,4] samples, and indicate the

0008-6215/86/$ 03.50

TABLE I

METHYLATION ANALYSIS OF CHRYSOLAMINARAN FROM *S. amphioxys* CULTURES AND A NATURAL DIATOM-DOMINATED COMMUNITY FROM THE UNDERSURFACE OF ANTARCTIC SEA ICE

O-Methyl derivative	*Deduced glycosidic linkage*	[*(mol %)*]	
		S. amphioxys	*Natural community*
2,3,4,6-Glc*p*	terminal	5	8
2,4,6-Glc*p*	3	88	86
2,3,4-Glc*p*	6	tr[a]	tr[a]
2,6-Glcp	3,4	1	0.5
4,6-Glc*p*	2,3	3	1
2,4-Glc*p*	3,6	3	4

[a]tr = <0.5%.

presence of (1→6)-inter-residue linkages. However, it is possible that trace levels of this tri-*O*-methylglucitol are artifacts arising from hydrolytic demethylation of terminal glucosyl groups[5], and may not be structurally significant.

The β-configuration of 3-linked glucosyl residues in the glucan was shown by hydrolysis with linkage-specific β-glucan hydrolases. (1→3)-β-D-Glucan exo- and endo-hydrolases completely hydrolyzed the chrysolaminaran to glucose or a series of gluco-oligosaccharides, respectively.

These results are similar to those found in previous studies on other diatom species[1–3] and indicate that the branching through O-2 may be a common structural feature of diatom chrysolaminaran.

The glucan extracted from the natural diatom community associated with the Antarctic sea ice has a similar linkage composition to that of *S. amphioxys* chrysolaminaran, with minor differences (Table I). Glucan accumulates as the ice communities reach stationary-growth phase, and as much as 60% of the photosynthetically fixed carbon may be directed into this material[6]. The cellular level of chrysolaminaran appears to be a useful parameter for measuring the physiological state of diatom communities[7]. In algal communities that produce copious mucilage polysaccharides, hot-water extraction followed by size fractionation is a convenient method for isolating a nearly pure preparation of chrysolaminaran.

EXPERIMENTAL

Material. — *S. amphioxys* was isolated (December, 1982) from the undersurface of sea ice near the Australian Antarctic station of Casey (66°17′S, 110°34′E) and grown under axenic conditions in f/2 medium[8], maintained at constant temperature (−1.2° ±0.4°) and irradiance (25 $\mu Em^{-2}s^{-1}$).

Cells were collected by centrifugation (3,800*g*, 20 min, 0°) and the pellet extracted with 10 mL of distilled water (2 h, 100°). The residue was removed by

centrifugation (1,400*g*, 15 min) and the extraction repeated (10 mL, 2 h, 100°). The combined supernatants were then size-fractionated by sequential ultrafiltration through a YM10 membrane (Amicon) and a YM2 membrane, with molecular-exclusion limits for globular molecules of 10,000 and 1,000, respectively. The retained fractions were then freeze dried prior to further analysis.

Analyses. — Carbohydrate content was determined by the method of Dubois *et al.*[9], using glucose as standard. The molecular weight of the glucan was determined by gel-permeation chromatography on a Hewlett-Packard 1084B liquid chromatograph equipped with a refractive-index detector. An Ultrapac TSK G2000 column (LKB, Sweden) (7.5 × 300 mm) with 10-μm bead size, was used. Chromatography was performed in 0.5M sodium phosphate buffer, pH 7.0, and the column calibrated against a series of polyethylene glycols of known molecular weight.

Hydrolysis was performed[10] in 2M trifluoroacetic acid (1 mL) for 2 h at 100° and the products successively reduced and acetylated by a modification of the method of Blakeney *et al.*[11] in which reduction was performed with sodium borodeuteride in diethylene glycol dimethyl ether.

G.l.c. was performed on a Hewlett–Packard chromatograph equipped with an on-column injector and flame-ionization detector. A SCOT glass-capillary column coated with Silar 10C (SGE, Melbourne, Australia, 25 m × 0.5 mm i.d.) was used with helium as carrier gas (0.9 mL/min flow rate). Samples in dichloromethane were injected directly onto the column at 38° and the oven ramped to 190° at 70°/min and then programmed from 190° to 230° at 3°/min and kept at 230° for 10 min.

G.l.c.–m.s. was performed with a fully automated Finnigan MAT 1020B g.l.c.–m.s. system (Sunnyvale, CA, U.S.A.) with a BP-75 vitreous silica WCOT column (SGE, Melbourne, Australia). Conditions were as described by Bacic *et al.*[12].

Methylation was performed by the method of Harris *et al.*[13] on 0.5 mg of polysaccharide. The permethylated, peracetylated alditols were analyzed by g.l.c. and g.l.c.–m.s.

Enzymic hydrolysis. — The polysaccharide was digested with two purified β-D-glucan hydrolases of defined linkage-specificities; a (1→3)-β-D-glucan exohydrolase (EC 3.2.1.58) from *Euglena gracilis*, and a (1→3)-β-D-glucan endohydrolase (EC 3.2.1.6) from *Rhizopus arrhizus*, kindly provided by Professor B. A. Stone, La Trobe University, Bundoora, Victoria 3083. Enzyme hydrolysis was conducted in 0.05M sodium acetate buffer, pH 5.5, with a final protein concentration of 0.2 mg/mL and polysaccharide concentration of 4 mg/mL. At zero time and after suitable incubation periods, samples (100 μL) were removed, heated in a boiling-water bath for 3 min, and deionized with a mixed-bed resin AG501-X8 (Bio-Rad). Descending p.c. was performed on Whatman No. 3 paper in 1-propanol–ethyl acetate–water (6:1:3 v/v/v) and sugars were detected with alkaline silver nitrate reagent[14]. Identification of products was by comparison with a

homologous series of β-linked gluco-oligosaccharides obtained by partial acid hydrolysis of laminaran.

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 B. S. PAULSEN AND S. MYKLESTAD, *Carbohydr. Res.*, 62 (1978) 386–388.
4 S. PEAT, W. J. WHELAN, AND H. G. LAWLEY, *J. Chem. Soc.*, (1958) 729–737.
5 W. D. ANNAN, E. HIRST, AND D. J. MANNERS, *J. Chem. Soc.*, (1965) 885–891.
6 M. J. MCCONVILLE, C. MITCHELL, AND R. WETHERBEE, *Polar Biol.*, 4 (1985) 135–141.
7 S. MYKLESTAD, *J. Exp. Mar. Biol. Ecol.*, 15 (1974) 261–274.
8 R. R. L. GUILLARD AND J. H. RYTHER, *Can. J. Microbiol.*, 8 (1962) 229–239.
9 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
10 P. ALBERSHEIM, D. J. NEVINS, P. D. ENGLISH, AND A. KARR, *Carbohydr. Res.*, 5 (1967) 340–345.
11 A. B. BLAKENEY, P. J. HARRIS, AND B. A. STONE, *Carbohydr. Res.*, 113 (1983) 291–299.
12 A. BACIC, P. J. HARRIS, E. W. HAK, AND A. E. CLARKE, *J. Chromatogr.*, 315 (1984) 373–377.
13 P. J. HARRIS, R. J. HENRY, A. B. BLAKENEY, AND B. A. STONE, *Carbohydr. Res.*, 127 (1984) 59–73.
14 W. E. TREVELYAN, D. P. PROCTER, AND J. S. HARRISON, *Nature (London)*, 166 (1950) 444–445.