The Reserve Polysaccharides of *Prototheca zopfii*

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The majority of protozoa synthesize either a starch-amylopectin-glycogen type polysaccharide or a β-(1→3)-glucan as a reserve carbohydrate (Manners & Ryley, 1963). We now describe the isolation of a mixture of polysaccharides from *Prototheca zopfii*, which include an unusual galactan.

The organism was grown at 24° and pH 5·4 for 7 days on a malt–agar medium; the cells were collected, stored in methanol, and later dried. The cell walls were extremely resistant, and could only be broken by grinding the dried cells with carborundum. The ground cells (60g.) were extracted successively four times with hot water to give 6·0g. of mixed polysaccharides (Fraction A). The residue was extracted thrice with hot 40% potassium hydroxide to give 2·2g. carbohydrate (Fraction B). Acid hydrolysis of both fractions gave a mixture of glucose, galactose and mannose.

Fraction A gave a brown stain with iodine, and on treatment with α-amylase, maltose and other sugars were released. An α-glucan was isolated by selective precipitation using iodine in 4% sodium chloride solution. This α-glucan (yield 0·33g.) had the properties of a typical glycogen, e.g. average chain length 14 glucose residues; β-amylolysis limit 49%; the successive action of isoamylase and β-amylase gave 73% conversion into maltose; the combined action of these enzymes gave 93% apparent conversion into maltose, thus establishing the presence of α-(1→6)-glucosidic inter-chain linkages.

The remainder of fraction A on acid hydrolysis gave D-galactose and D-mannose in the ratio of 26:1. This galactan was water-soluble and had $[\alpha]_D$ +41°. On methylation and hydrolysis the products were tetra-*O*-methyl-D-galactose (10%), tri-*O*-methyl-D-galactose (73%) and di-*O*-methyl-galactose (16%). Subsequent analysis showed that the tetra-*O*-methyl fraction was a mixture of pyranose and furanose sugars, and the tri-*O*-methyl fraction contained largely the 2,3,4-isomer, with smaller amounts of the 2,4,6-isomer.

On oxidation at 2°, the galactan reduced 1·42mol. prop. of periodate and released 0·10mol. prop. of formaldehyde. At 18°, 1·63mol. prop. of periodate was reduced and 0·66mol. prop. of formic acid was released. These results indicate the presence of a substantial proportion of (*a*) (1→6)-linked D-galactopyranose residues, (*b*) D-galactofuranose end-groups. The latter was confirmed by partial acid hydrolysis; about 10% of the galactan was readily released by 0·1N-sulphuric acid within 30min.

The galactan has therefore a branched structure with main chains of (1→6)-linked D-galactose residues and side chains containing terminal D-galactofuranose residues.

Fraction B contained a mixture of the glycogen, the galactan and a (1→4)-linked mannan.

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The Enzymic Degradation of Laminarin

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In continuation of structural studies on laminarin (Fleming & Manners, 1965; Fleming, Hirst & Manners, 1966), we now report the results of an enzymic degradation analysis.

Incubation of laminarin with a bacterial laminarinase preparation (Manners & Patterson, 1966) resulted in slow random degradation with the production initially of glucose, laminaribiose, laminaritriose and other sugars. The laminaritriose was later hydrolysed to glucose and laminaribiose. In one experiment with soluble laminarin, the increase in reducing power after 5, 6, and 10 days incubation at 40° corresponded to 55, 56 and 60% apparent conversion into glucose; with insoluble laminarin, the extent of hydrolysis was 62, 65 and 73% respectively.

Soluble laminarin from *Laminaria saccharina* (2·0g.) was incubated with the enzyme preparation until the apparent conversion into glucose was 60%. Part of the hydrolysate was deionized, concentrated and fractionated by preparative paper chromatography. Fractions corresponding to glucose, laminaribiose (14·8mg.), two reducing oligosaccharides (I and III), two non-reducing oligosaccharides (II and IV) and a mixture of higher oligosaccharides (26·5mg.) were collected.

Fraction II, yield 4·4mg., gave glucose and mannitol on acid hydrolysis, and had the same chromatographic properties as laminaribiosyl-mannitol.

Fraction IV, yield 3·5mg. was also composed of glucose and mannitol, and had the same chromatographic properties as β-glucosylmannitol.

Fraction III, yield 12·6mg. did not contain

mannitol, and on partial acid hydrolysis gave a mixture of glucose, laminaribiose and gentiobiose. After borohydride reduction of fraction III, a partial hydrolysate contained glucose, sorbitol, gentiobiose and laminaribi-itol. Fraction III was therefore 6^2-β-glucosyl-laminaribiose.

Fraction I yield 9·4mg., on partial hydrolysis gave rise to fraction III, and the same products as fraction III. This suggests that fraction I is a tetrasaccharide structurally related to fraction III. The presence of a (1→6)-linked glucose residue in fraction I (and hence, in the original sample of laminarin) was shown by micro-methylation followed by gas–liquid chromatography. The methanolysate of methylated fraction I contained the methyl glycosides of 2,3,4,6-tetra-, 2,4,6-tri- and 2,3,4-tri-*O*-methyl-D-glucose.

The results show that the enzyme preparation is able to bypass (1→6)-inter-chain linkages in branched β-(1→3)-glucans, but cannot hydrolyse certain (1→3)-linkages which are adjacent to the inter-chain linkage or, in the case of laminarin, to a non-reducing mannitol end-group.

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