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Structural Studies of a Glucan Isolated from Blue–Green Alga *Spirulina platensis*

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ABSTRACT

The acid-soluble polysaccharide isolated from Spirulina *contained glucose, principally, with small amounts of sulphate. The latter was removed by passing through a DEAE-cellulose column. Methylation analysis, periodate oxidation, chromium trioxide oxidation, oligosaccharide and enzymatic studies revealed the polymer to have an α-1,4 linked glucan backbone with frequent side chains through α-1,6 linked glucosyl residues.*

INTRODUCTION

Blue–green alga, *Spirulina platensis*, is receiving world wide attention as an unconventional source of protein for use in feeds and foods (Venkataraman *et al.*, 1980). While a considerable amount of data is available on the proteins and nutritional and toxicological evaluation of *Spirulina* (Anusuya Devi & Venkataraman, 1984), not much data are available on carbohydrates. Different classes of algae have different reserve polysaccharides. These include starch, glycogen, laminaran (β-1,3 linked glucan), floridan starch (hybrid of laminaran and glycogen) and inulin (α-2,1 linked fructan) (Painter, 1983). This report deals with the chemical nature of a glucan isolated from *Spirulina*.

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MATERIALS AND METHODS

Cultivation of alga

Spirulina platensis was cultivated as described by Venkataraman *et al.* (1980), in fresh water with bicarbonate (8 g/litre) as the carbon source, and sodium nitrate (1·5 g/litre) as the nitrogen source. The other nutrients used in the medium were K_2HPO_4 (0·5 g/litre), NaCl (1·0 g/litre), $MgSO_4.2H_2O$ (0·2 g/litre), K_2SO_4 (1·0 g/litre), $CaCl_2.2H_2O$ (0·04 g/litre) and $FeSO_4.7H_2O$ (0·01 g/litre). The alga was harvested by filtration, washed thoroughly with water until it was free from salts and freeze-dried.

General methods

All evaporations were done at reduced pressure at a temperature of below 40°C. Total sugar and uronic acid estimations were done by phenol-H_2SO_4 (McKelvy & Lee, 1969) and carbazole (Knutson & Jeanes, 1968) methods, respectively. Paper chromatography was done on either Whatman No. 1 or No. 3 papers using *n*-propanol–ethanol–water (7:1:2, v/v) as a solvent system. The chromatograms were sprayed with either $AgNO_3$/NaOH (Trevelyan *et al.*, 1950) or aniline–phthalate (Partridge, 1949) reagents. Sulphate content in the polymer was determined by the turbidometric method (Sperber, 1948). Electrophoresis of polysaccharide was done after dyeing the polysaccharide with proceon dye (Anderson *et al.*, 1971) on a cellulose acetate membrane using a Beckman microzone electrophoresis unit, model 101 with ammonium carbonate buffer (0·05M, pH 9·3) at an applied voltage of 180 V. Gel permeation chromatography was done on Sephacryl S-200 or Biogel P-60 columns using standard dextrans (T-10, T-20, T-40 and T-70). DEAE-cellulose column chromatography was done as reported earlier (Salimath & Tharanathan, 1982). Optical rotation was measured on a Carl-Zeiss polarimeter using 1% aqueous solution at room temperature. The polysaccharide was hydrolysed with 2N trifluoroacetic acid (TFA) at 100°C for 6 h. GLC analysis was done on a Packard model 437 gas chromatograph equipped with a flame ionization detector using OV-225 or ECNSS-M column. GLC–MS was done on a Finnigan model MAT 1020 B coupled to a model 6000 data and graphic output, with an electron energy of 70 eV, ion source temperature of 50°C, mass range (m/z) 40–600 and integration time of 7 ms per scan. The column used was a CP-Sil 5 at a column temperature of 70–220°C with a programme rate of 2°C/min and the carrier gas used was helium.

Extraction of the acid-soluble polysaccharide

The defatted algae was sequentially extracted with 70% alcohol and cold and hot water. The insoluble residue was extracted with HCl (pH 2·0) at 70°C (X3, 8 h). The extracts were pooled, dialysed, concentrated, precipitated with alcohol (3 volumes) and dried.

Methylation analysis

Polysaccharide (~2 mg) was methylated by the method of Hakomori (1964). Complete methylation of the polysaccharide was ascertained by IR spectrometry. The resultant fully methylated polysaccharide was purified by washing with water followed by Sephadex LH-20 column chromatography. The sample was then hydrolysed, reduced ($NaBD_4/D_2O$) and acetylated for GLC and GLC–MS analysis.

Periodate oxidation

The polysaccharide (10 mg) was dissolved in water (10 ml) and oxidized by adding 2 mM sodium metaperiodate (10 ml) and the reaction mixture was left at +4°C in the dark. Aliquots were taken at different time intervals and periodate consumed (Fleury & Lange, 1933) and formic acid released (Brown *et al.*, 1948) were estimated.

CrO_3 oxidation

The polysaccharide (10 mg) was acetylated with acetic anhydride (2 ml) in the presence of pyridine (2 ml) and formamide (8·9 ml) for 16 h. After the reaction was completed excess reagents were removed and the acetylated polysaccharide was taken in glacial acetic acid (2·5 mg) and oxidised with chromium trioxide (125 mg) for 12 h (Salimath & Tharanathan, 1982). The mixture and the control (without chromium trioxide) were purified, hydrolysed (2N TFA 100°C, 8 h) and analysed by PC and GLC.

Enzymatic studies

The polysaccharide (~5 mg) was treated with different amylolytic enzymes and, after the reaction was over, alcohol (3 volumes) was added, centrifuged and the supernatant was taken for paper chromatographic analysis. The conditions used were (a) α-amylase, 1 mg, phosphate buffer (pH 6·9, 0·05M) (MacGreger & Ballance, 1980); (b) α-glucosidase, 1 mg, acetate buffer (pH

4·8, 0·05M, 60°C) (Hayashida *et al.*, 1982); (c) pullulanase, 0·1 ml, citrate buffer (pH 5·0, 0·05M, 37°C) (Ueda & Marshall, 1980).

RESULTS AND DISCUSSION

The acid-soluble polysaccharide isolated from *Spirulina* contained mainly glucose (97%) and had 4·9% sulphate. The latter could not be dissociated from the glucan either by alcohol precipitation or by dialysis, but was done by passing through a column of DEAE-cellulose, suggesting that the sulphate groups are not covalently bound and that this is a glucan-type of polymer. The glucan gave a reddish-brown colour by the starch iodine test. Chemical characteristics of the acid-soluble polysaccharide and the purified glucan are given in Table 1.

The polysaccharide was found to be homogeneous by sedimentation, electrophoresis and gel permeation chromatography (Salimath & Tharanathan, 1982). Molecular weight determined on Biogel P-60 and Sephacryl S-200 showed $\bar{M}w$ of 3800. The polymer was methylated by the Hakomori method (Hakomori, 1964) and was hydrolysed, reduced, acetylated and subjected to GLC and GLC–MS analysis (Table 2). Three peaks observed were identified as 2,3,4,6-tetra-*O*-methyl-D-glucose (16·4%), 2,3,6-tri-*O*-methyl-D-glucose (69·3%) and 2,3-di-*O*-methyl-D-glucose (13·8%). This suggests that the glucan is branched having 1,4 linkages in the main chain with branches through 0–6.

The glucan consumed 1·04 mole of periodate (Table 3) and liberated 0·14 mole of formic acid per mole anhydrosugar. The periodate-oxidized product, upon complete hydrolysis, gave glycerol and erythrytol.

When the polysaccharide was subjected to partial acid hydrolysis, a number of oligosaccharides, besides glucose, were seen on the chromatogram. Two disaccharides observed had R_f values similar to maltose and

TABLE 1
Chemical Characteristics (%) of ASP and Glucan

	ASP	*Glucan*
Yield	(100)	90·0
Total sugar	79·9	82·2
Glucose	97·4	98·2
Sulphate	4·9	–
$[\alpha]_D$	+165°	+160°

ASP, Acid-soluble polysaccharide.

TABLE 2
Methylation Analysis of Glucan

Peak number	O-*methyl ether*	T_{TMG}	*Diagnostic mass fragments (m/z)*	*Mode of linkage*	*% Concentration*
1	2,3,4,6-Me_4-Glc	1·0	205, 162, 161, 145, 118, 101, 45	$[Glc_p] \rightarrow 1$	16·4
2	2,3,6-Me_3-Glc	2·3	233, 162, 118, 113, 102, 45	$\rightarrow 4[Glc_p] \rightarrow 1$	69·8
3	2,3-Me_2-Glc	4·5	261, 201, 162, 118, 102	$\rightarrow 4[Glc_p] \rightarrow 1$ ↑ 6	13·8

isomaltose and the trisaccharide to maltotriose. Other oligosaccharides were not identified further due to insufficiency of the material.

The glucan was subjected to α-glucosidase, α-amylase and pullulanase and the sugars released were identified by PC as glucose and maltose.

CrO_3-oxidation of the polymer did not destroy glucose residues, indicating their axial position and that the glucosyl residues are α-linked. The $[\alpha]_D$ value of $+165^\circ$ (water) was also in agreement.

Thus the glucan has a main chain of α-1,4 linked glucose residues and is branched through glucosyl residues by α-1,6 linkages, suggesting it to be a branched glycogen type of polymer, and is likely to be a reserve polysaccharide of *Spirulina.*

Blue–green algae and bacteria are similar in their cellular organization and biochemical properties. Bacteria synthesise glycogen as a reserve product under suitable culture conditions. Glycogen is built up in the stationary phase of growth when the carbon source is in excess in blue–green algae. In this study *Spirulina* was cultivated (Venkataraman *et al.*, 1980) in a nutrient medium of high carbon source (8 g $NaHCO_3$ per litre culture)

TABLE 3
Kinetics of Periodate Oxidation of Glucan

Time (h)	*Mole of periodate consumed per anhydro sugar*
0	—
8	0·59
24	1·04
36	1·04

against an optimum dose of 4·5 g and was harvested at stationary phase (OD, 0·6) to obtain a reproducible yield of glycogen. A preliminary account of the identification of glycogen (0·5%) in *Spirulina* is reported (Santillan, 1982), but no systematic work was done. Glycogen is also reported in *Anacystis nidulens* (Weber & Wober, 1975), *Nostoc muscorum*, (Chao & Bowen, 1971) and *Anabaena variabilis* (Weckesser *et al.*, 1974).

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