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STRUCTURE OF AN α-D-GALACTOSAMINOGLYCAN FROM *Physarum polycephalum* SPHERULE WALLS

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ABSTRACT

The spherule walls of *Physarum polycephalum* have been reexamined and found to contain 88% of galactosamine (as anhydrogalactosamine), 6.8% of protein, 4.7% of phosphate groups, and a small proportion of acetyl groups (0.5%). Methylation studies indicated that the spherule-wall polysaccharide is a long-chain galactosaminoglycan linked exclusively (1→4) and without phosphate linkages. The specific optical rotation of this unique glycan, $[\alpha]_D$ +118° (6M HCl), indicated that it is α-D-linked.

INTRODUCTION

The myxomycete *Physarum polycephalum* produces hard-walled sclerotia (spherules) under certain conditions, such as nutrient depletion. It has been shown previously that the spherule wall is composed almost entirely of D-galactosamine residues[1]. Galactosaminoglycans are not commonly found in Nature. Such polymers have been detected as minor components in cell walls of a number of Ascomycetes[2]. A galactosaminoglycan has been detected in the culture fluid of *Aspergillus parasiticus*[3] and in the cell walls of *A. niger*[4]. Galactosamine was also found to be a major constituent of the cell wall of a virus-containing strain of *Penicillium stoloniferum*[5]. This communication describes the structural characterization of an α-D-galactosaminoglycan isolated from *P. polycephalum* spherule walls.

EXPERIMENTAL

Culture conditions and isolation of Physarum polycephalum *spherule walls.* — Microplasmodia of *P. polycephalum* were obtained and cultivated as described previously[6]. Spherules were obtained either from nutrient-exhausted cultures (2 weeks old) or by inducing spherulation by transferring 48-h old microplasmodia to an equal volume of minimal-salt solution[7]. Spherulation started after 24 h and was completed after 36–48 h. The spherules were centrifuged off from the culture fluid (1.2 l) at 4°, washed extensively with water, and disintegrated with an X-Press (AB Biox, Sweden) or by sonication with a Branson Sonifier B 12 (3 × 15 min at 0°).

Clean wall preparations (112 mg) were then obtained by extensive washing of the suspension according to McCormick *et al.*[1].

Analytical methods. — The optimal conditions for the acid hydrolysis of spherule walls were 6M HCl, 100°, 18 h. Total amino sugars were determined by a modified Levvy–McAllen method[8] with D-galactosamine as the standard.

Paper chromatography was performed with the following solvent systems: (A) 2:5:7 (v/v) pyridine-ethyl acetate–water (upper phase)[9] and (B) 9:5:4 (v/v) butyl alcohol–pyridine–water containing 0.5% of acetic acid[10]. Sugars and amino sugars were revealed with the ammoniacal silver nitrate reagent[11] and ninhydrin, respectively

The phosphate content was determined according to Chen *et al.*[12] and the protein content according to the method of Lowry *et al.*[13]. For the determination of acetyl groups, the spherule walls were hydrolyzed[14] for 2 h at 100° with 2M HCl (6 mg/ml), and acetic acid was determined[15] by g.l.c. on a SP-1200-Chromosorb W-AW column (4.2 m) operated at 110°.

Methylation procedure. — The spherule walls (5 mg) were methylated by the Hakomori procedure as modified by Sandford and Conrad[16]. The methylated polysaccharide was acetolyzed and hydrolyzed as described by Stellner *et al.*[17]. The resulting, methylated amino sugars were reduced, acetylated[18], and analyzed by g.l.c. and m.s. G.l.c. was performed on a column[18] of 3% of ECNSS-M coated on Gas Chrom Q at 175°. M.s. was performed with an AEI MS9 mass spectrometer operated at 70 eV and 100 μA with a source temperature of 100°. The spectrometer was coupled to an Instem DATAMASS data system. Samples were introduced with the direct insertion probe.

Optical rotation measurement. — The purified spherule walls (20 mg) were dissolved in 6M HCl (2 ml) at 25°. After 30 min, a trace of undissolved material was sedimented by centrifugation. An aliquot of the supernatant liquor was analyzed for galactosamine after total acid hydrolysis. The optical rotation of the remainder was followed as a function of time. The optical rotation of the polysaccharide was extrapolated to time 0, and the specific rotation calculated from the total anhydro-galactosamine content.

Brightener treatment. — The spherule walls were treated with Calcofluor white M2R (American Cyanamid Co., Bound Brook, New Jersey 08805, U.S.A.) as described by Maeda and Ishida[19], and examined by fluorescence microscopy.

RESULTS AND DISCUSSION

The presence of D-galactosamine as the only sugar present in *Physarum polycephalum* spherule walls, reported by McCormick *et al.*[1], was confirmed by paper chromatography of the acid hydrolyzate of the purified walls in solvents A and B, and by ninhydrin degradation[10], which yielded only lyxose (solvent C). No other sugars could be detected in the hydrolyzate. The walls were found to contain 88% of D-galactosamine (as anhydrogalactosamine), 6.8% of protein, 4.6% of phosphate, and 0.5% of acetyl groups (molar ratio of acetyl groups to D-galactosamine, 1:66).

The galactosaminoglycan was completely methylated in one step. After hydrolysis and reduction, the only peak detected by g.l.c. corresponded to 1,5-di-*O*-acetyl-2-deoxy-2,3,6-tri-*O*-methyl-2-(*N*-methylacetamido)galactitol. Its retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol was 5.30 and its identity was established by mass spectrometry. This showed that the galactosaminoglycan was linked exclusively (1→4). Since no 1-*O*-acetyl-2-deoxy-2,3,4,6-tri-*O*-methyl-2-(*N*-methylacetamido)galactitol (arising from the nonreducing end unit) could be detected, the average degree of polymerization of the polysaccharide is probably high.

In contrast to the findings of McCormick *et al.*[1], the galactosaminoglycan was not soluble in M HCl, and solubilization could only be achieved in 6M HCl. The reason for this discrepancy is not known, but could be attributed to the lower purity of the polysaccharide isolated by McCormick *et al.*[1], which contained more phosphate groups and protein, or to a different mode of linkage of the galactosamine units. The optical rotation of the polysaccharide decreased as a function of time and its specific optical rotation $[\alpha]_D^{25}$ +118° (*c* 5, 6M HCl) indicated that the D-galactosamine residues were α linked. This was confirmed by treatment of the walls with the brightener Calcofluor white M2R which binds specifically to polysaccharides having a β-D configuration[19]. Only an insignificant fluorescence was observed.

The galactosaminoglycan of *P. polycephalum* spherule walls is therefore a long-chain polymer linked α-D-(1→4) and it is acetylated to only a small extent. As already indicated by McCormick *et al.*[1], the presence of phosphate groups (4.6%) could be an artifact of extraction. Under the condition of methylation used, the phosphate groups of a glycan phosphate would be alkali resistant and lead to a partially methylated polysaccharide. However, the galactosaminoglycan was completely methylated.

Zaar and Kleinig[20] have discussed the sequence of ultrastructural events leading to spherulation. They describe the spherule wall as consisting of two layers of fibrils with different staining properties. The results described here indicate that the purified walls are composed almost entirely of D-galactosamine residues. However, the loosely attached outer layer could have been easily removed during the extensive purification procedure. *P. polycephalum* produces a viscous extracellular polysaccharide that was shown to be a D-galactan, probably β linked[21], and it could be analogous to this outer slime layer.

The mechanism involved in the switching over from the production of an extracellular β-D-galactan to that of an α-D-galactosaminoglycan of the spherule cell wall within a few hours has yet to be elucidated.

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