

Note

Composition and partial structure of the extracellular polysaccharide of *Physarum polycephalum*

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(Received April 20th, 1972, accepted for publication May 17th, 1972)

The extracellular slime produced by the myxomycete *Physarum polycephalum* is known to be a polysaccharide and has been the subject of preliminary investigations^{1,2} The slime has been reported to be a sulfated galactan containing trace amounts of rhamnose² Direct sulfate analysis indicated a ratio of sulfate to galactose of about 1.4

In this study, *Physarum polycephalum* was grown in a semidefined medium, and the purified slime was found to be a D-galactan, partially substituted by sulfate and phosphate groups with a DP of 128 Analysis indicated a ratio of phosphate and sulfate to galactose of 1.14 and 1.21, respectively No trace of rhamnose could be detected In view of low specific optical rotation ($[\alpha]_D^{25} + 16^\circ$) the polysaccharide is probably β -D-linked Attempts to remove sulfate and phosphate groups were not successful

The polysaccharide was resistant to complete methylation, and the partially methylated polysaccharide also contained sulfate and phosphate groups Analysis of the cleavage products, however, indicated the presence of (1→3)-, (1→4)-, and (1→6)-

TABLE I

PRODUCTS OF HYDROLYSIS OF THE METHYLATED POLYSACCHARIDE OF *Physarum polycephalum*^a

O-Methyl-D-galactitol acetates	Molar proportion	T values ^b			
		Column A		Column B	
		Found	Lit ^{1,2}	Found	Lit ^{1,2}
2,3,4,6-Tetra-	1.0	1.25	1.25	1.19	1.19
2,4,6-Tri-	0.9	2.32	2.28	2.02	2.03
2,3,6-Tri-	2.7	2.49		2.20	
2,3,4-Tri-	1.4	3.55	3.41	2.92	2.89
2,6-Di-	2.0	3.78	3.65	3.20	3.14
3,6-Di-	traces	4.49		3.71	
2,3-Di-	4.9	6.21		4.85	
2,4-Di-	3.0	6.75	6.35	5.20	5.10
2-Mono-	0.3	8.68		6.45	

^aAfter reduction and acetylation ^bRelative to 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-D-glucitol

linked D-galactose units (Table I) The polysaccharide is branched as judged by the high content of 2,3,4,6-tetra-O-methyl-D-galactose However, the mode of linkage of the branches is not known because of the presence of a large proportion of di-O-methyl-D-galactoses arising either from the branching points or from the phosphate- and sulfate-substituted D-galactose units

Preliminary results indicated a similar structure for the slime of two other strains of *Physarum polycephalum*

EXPERIMENTAL

General methods — The total sugar content of the polysaccharide was estimated by the phenol-sulfuric acid method without preliminary acid hydrolysis, using D-galactose as a standard³ Reducing end-groups were determined by a modified Park and Johnson method⁴ Neutral sugars were characterized by paper chromatography after acid hydrolysis (M sulfuric acid, 100°, 4 h) using the following solvent systems (A) 2.5:7 pyridine-ethyl acetate-water (upper phase)⁵, and (B) 9.5:4 butyl alcohol-pyridine-water Sugars were revealed by the ammoniacal silver nitrate reagent⁶

Acetyl groups were determined by the method of Hestrin⁷, phosphate by the method of Chen⁸, and sulfate by the method of Dodgson and Price⁹

Methylation of the polysaccharide was performed by a modified Hakomori method¹⁰ The individual methyl sugars were converted into their alditol acetates¹¹ and analyzed by g l c and m s

G l c was performed at 180° with a glass column (200 × 0.3 cm) containing 3% (w/w) of ECNSS-M (column A) or 3% OV-225 (column B) on Gas Chrom Q (100–120 mesh)¹² The relative concentration of individual sugars was calculated assuming that the response factor is proportional to the molecular weight of the acetylated O₇-methylalditols¹³ Combined g l c and m s was performed on column B with a Pye 104 gas chromatograph coupled to an AEI MS 20 mass spectrometer

Purification of the polysaccharide — *Physarum polycephalum* was obtained from Dr R Braun, ISREC, Lausanne (Switzerland) Plasmodia were grown in submerged culture as described by Daniel and Baldwin¹⁴ until maximal slime production occurred (4–5 days) After removal of the plasmodia by centrifugation at 500 g for 5 min, the polysaccharide was precipitated from the supernatant with ethanol (2 vol.) Precipitation was also effected by the cetyl pyridinium chloride method of Scott¹⁵ Identical materials were obtained after repeated precipitations with ethanol by use of either methods (yield, 0.5 g/l of growth medium), $[\alpha]_D^{25} + 16^\circ$ (c 0.5, water) The polysaccharide was found to be homogeneous by cellulose acetate electrophoresis² at pH 8.5 It migrated toward the anode The viscosity of a 0.5% solution in water was measured in an Ostwald-Fenske viscosimeter at 20° and found to be 2.26 centipoises

Composition of the polysaccharide — Examination of the acid hydrolyzate of the polysaccharide by paper chromatography (solvents A and B) revealed only

galactose The presence of D-galactose was confirmed by enzymic identification using D-galactose dehydrogenase (D-galactose NAD oxidoreductase, E C 1 1 1 48) The polysaccharide was shown to contain 91% D-galactose (as anhydro-D-galactose), and was analyzed for the presence of nitrogen (1 55%), phosphate (4 15%), and sulfate (2 81%) groups No acetyl groups were detected The average degree of polymerization (DP) was found to be 128 by reducing end-group analysis

Methylation of the polysaccharide — The polysaccharide was methylated¹⁰ and emulsified with 1 1 chloroform–water After centrifugation, the methylated polysaccharide was collected at the interphase and washed three times with 1 1 chloroform–water, and then freeze-dried The methylated polysaccharide was hydrolyzed with M sulfuric acid for 4 h at 100°, and the methylated sugars were converted into their alditol acetate derivatives and analyzed by g l c and m s The methylation was repeated, and the molar proportions of methylated galactitol acetates were found to be unchanged and are given in Table I The methylated polysaccharide was analyzed for phosphorus (1 0%) and sulfur (1 8%)

Attempts to desulfate the polysaccharide with methanolic hydrogen chloride¹⁶ failed to reduce the sulfate and phosphate content The polysaccharide was also treated for 11 h at 80° with 2 5M sodium hydroxide–M sodium borohydride After neutralization and dialysis, the material was freeze-dried and analyzed for phosphorous (2 62%) and sulfur (1 28%) The material was methylated twice, but no significant difference in the molar proportions of the methylgalactitol acetates was found.

ACKNOWLEDGMENT

The authors wish to thank Dr I Horman for the interpretation of the mass-spectrometry data

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