Phytochemistry, 1975, Vol. 14, pp. 2459–2461. Pergamon Press. Printed in England.

POLYSACCHARIDE FROM CELL WALLS OF *CHLAMYDOMONAS REINHARDTII*

KUO-SHII JIANG and GEORGE A. BARBER

Department of Biochemistry, Ohio State University, 484 West 12th Ave., Columbus, Ohio 43210, U.S.A.

(*Received* 8 *February* 1975)

Key Word Index—*Chlamydomonas reinhardtii* Family; alga; cell wall; polysaccharide.

Abstract—The cell wall fraction of the green alga *Chlamydomonas reinhardtii* contained about 25% carbohydrate after prolonged treatment with salivary amylase. A polysaccharide extracted with strong alkali and purified by gel filtration had a MW of 41 500 and contained the molar proportion of monosaccharides: arabinose 3·3; mannose 1·0; galactose 3·5; glucose 0·8. Galactose in the polymer was shown to consist of a mixture of the D and L enantiomers in a ratio of 84:16. A water-soluble polysaccharide was also isolated from the cell homogenate and was made up of the following molar proportion of monosaccharides: rhamnose 1·0; arabinose 1·4; mannose 3·4; galactose 2·3; glucose 1·0. Partial acid hydrolysis of alkali-insoluble material of the cell walls produced cellodextrin oligosaccharides similar to those derived from cellulose.

INTRODUCTION

In this paper we report the isolation, purification and characterization of a polysaccharide from the cell wall of the green alga *Chlamydomonas reinhardtii*.

RESULTS

The cell wall preparation, after extraction with EtOH and Et_2O, was found to contain by weight 28% carbohydrate [1], 24% protein [2], and 3% phosphate [3] as measured colorimetrically. Of the total weight, 22% was obtained as ash. The French cell homogenate of the algae contained in addition to the insoluble cell-wall material a small amount of a water-soluble compound that was precipitated by acetone. (Yield 68 mg from 60 g fr. wt of alga). It is 92% carbohydrate and presumably a polysaccharide. It was shown to have the following molar proportions of monosaccharides: rhamnose 1·0, arabinose 1·4, mannose 3·4, galactose 2·3, glucose 1·0.

Analysis of the alkali-soluble material of the wall revealed 78% carbohydrate which represents about 33% of the total carbohydrate in the wall fraction. This material was further purified by molecular sieve chromatography through columns of Bio-gel P-200 and Sepharose 6B. The compound eluted from Sepharose was subjected to ultracentrifuge analysis and an aqueous solution of the polysaccharide sedimented as a single peak at 40000 rpm. The rate of movement of the compound at 8000 rpm, assuming $\bar{v} = 0{\cdot}65$ [4], was used to calculate a MW of 41 500 [5]. Molar ratios of the monosaccharide components were arabinose 3·3, mannose 1·0, galactose 3·5, glucose 0·8. Since both the D and L enantiomers of galactose have been found in algal polysaccharides [6] the configuration of the galactose in the alkali-soluble polysaccharide of *Chlamydomonas*, was determined after hydrolysis and chromatography by reaction with D-galactose dehydrogenase [7]. The D-enantiomer comprised 73% of the galactose. The validity of this determination was supported by the optical rotation of the purified galactose isolated from the compound, $[\alpha]_D^{25} = +55°$ (c 0·45); this rotation was calculated to be from a 84: 16 mixture of the D- and L-enantiomers ($[\alpha]_D = +79°$ and $-79°$ respectively).

A sample (15 mg) of the alkali *insoluble* material after treatment with amylase and pronase was heated at 100° for 60 min in 38% HCl. Aliquots were chromatographed both in the descending direction and radially and a group of glucose-containing compounds appeared that were identical in R_f value to those obtained by hydrolysis and chromatography of cellulose under identical conditions. Several glucosyl oligosaccharides other than those of the cellodextrin series were also present.

DISCUSSION

The preparation designated here as 'cell wall' undoubtedly also contains material from other parts of the cell. For example, the high ash content (22%) probably represents the deposition of mineral salts elsewhere in the cell and their inclusion in the insoluble fraction obtained upon centrifugation of the cell homogenate. Similarly, in the wall, the starch-like substance that disappeared on treatment with salivary amylase was probably derived from granules or plastids sequestered elsewhere in the cell. Nevertheless, the alkali-soluble polysaccharide isolated from *Chlamydomonas* has the expected properties of a structural component and is thought to be a unit of the cell wall. It is similar to the alkali-soluble polysaccharide isolated from a strain of *Chlorella pyrenoidosa* [7] particularly with regard to the proportions of D- and L-galactose.

The wall of *C. reinhardtii* is reported to be made up of a lattice of glycoproteins sandwiched between two more variable fibrillar layers of the wall [9,10]. If this is true, the polysaccharide described here may be a component of one of those fibrillar layers. The soluble polysaccharide found in the growth medium may be another component of those layers sloughed off during cell multiplication. Such extracellular polysaccharides are apparently produced by many species of *Chlamydomonas* [11].

An examination of *Chlamydomonas* wall by X-ray diffraction failed to indicate the presence of cellulose microfibrils [12] and Roberts *et al.* [10] were unable even to find glucose in hydrolysates of their wall preparations. On the basis of our results, if cellulose is present, it is certainly not in any great quantity, and the polymer may be a glucan containing other linkages in addition to the β-1,4 of cellulose. However, there does appear to be a small amount of a polymer or polymers in the wall with some of the chemical properties of cellulose.

EXPERIMENTAL

Chromatography. GLC was carried out at 200° using a 2 m glass column containing 3% OV225 on Chromosorb W. Monosaccharide analyses were carried out essentially as described by Albersheim, *et al.* [13] PC was performed in a descending direction on Whatman No. 1 paper. The following solvent systems were used: Solvent I, *n*-PrOH–EtOAc–H_2O (7:1:2); Solvent II, C_5H_5N–EtOAc–HOAc–H_2O (5:5:1:3); Solvent III, C_5H_5N–EtOAc–H_2O (5:12:4). Carbohydrates were detected with $AgNO_3$ spray reagent [8].

Growth of algae. C. reinhardtii, + and − strains No. 89 and No. 90 (Culture Collection of Algae, Department of Botany, Indiana University, Bloomington) were maintained aseptically on a high Mg-salts-agar medium [14] in dim light. Cells from two large slants (2·5 × 15 cm) were used to inoculate aseptically 12 l. of the medium [14] in a fermentor. The medium was maintained at 25°, agitated at 180 rpm, gassed through a sparger with 1·5% CO_2 in air and illuminated with a semicircular bank of twelve 15 W bulbs at about 5 cm from the surface of the fermentor vessel. The culture was allowed to grow to the stationary phase (about 5 days). Growth of the culture was determined by counting the cells in a hematocytometer. Algae were harvested in a centrifuge at room temp. and washed 2× with H_2O in a centrifuge at 1500 *g*. The yield of cells was 1·5–2·0 g fr. wt per l. of medium. Centrifugal pellets were frozen and stored at −40°.

Isolation of algal cell walls. In a typical preparation, 60 g of the frozen cells were thawed, suspended in 40 ml of H_2O in a glass homogenizer with a Teflon pestle and passed twice through a French pressure cell at 15–20000 psi. The supernatant soln was reserved for later analysis. Residue was washed 2× by suspending it in H_2O and centrifuging at 20000 *g* for 20 min. It was then washed several times with 95% EtOH until the green color had disappeared and then dried *in vacuo*. The centrifugal supernatant soln was heated at 85° for 10 min to denature protein, filtered, concentrated to a small vol in a rotary evaporator at 40° and poured into 4 vol of Me_2CO with stirring. The ppt. was washed with Me_2CO and Et_2O and air dried (68 mg). It was then treated with α-amylase to remove starch and with pronase to remove protein as follows: The material was suspended in 10 ml of 0·1 M NaPi buffer, pH 7, 1 ml of crude human salivary α-amylase [15] was added, and the mixture was incubated 18 hr at 37°. The soln was poured into 4 vol of EtOH and the ppt recovered by centrifugation and air dried. It was then suspended in 10 ml of 5 mM Tris·HCl, pH 7·3, containing 6 mg of pronase and incubated 18 hr at 20°. Residue was recovered by precipitation from 4 vol of EtOH and dried in air.

Alkali extraction of a polysaccharide from the cell wall fraction. Dried cell wall preparation (900 mg) was stirred for 2 hr at 20° with 20 ml quantities of 4 M KOH containing 30 mM $NaBH_4$ under 1 atm. of N_2. The procedure was repeated × 3. The 3 suspensions were combined and insoluble material was removed by centrifugation, washed with dil KOH and H_2O and dried *in vacuo* (80 mg). The alkaline supernatant soln was neutralized with 50% HOAc. No material precipitated from soln at neutral pH. Upon addition of 4 vol of EtOH to the

soln, a ppt formed which was air dried and treated with amylase and pronase to remove protein and starch as described above. Final yield of alkali-soluble material was 109 mg (12% cell wall fraction). About 60% of this material dissolved upon subsequent suspension in buffer.

Acknowledgment—This research was supported in part by NIH Grant No. GM 19701. Dr. Gary Means kindly provided assistance with some of the ultracentrifugation.

REFERENCES

1. Hodge, J. E. and Hofreiter, B. T. (1962) in *Methods in Carbohydrate Chemistry* (Tipson, R. S. and Horton, D., ed.) Vol. I, pp. 380–394 Academic Press, New York.
2. Rock, A. L. and Putnam, S. L. (1971) *Anal. Biochem.* **44**, 239.
3. Ames, B. N. and Dubin, D. T. (1960) *J. Biol. Chem.* **235**, 796.
4. Olaitan, S. A. and Northcote, D. H. (1962) *Biochem. J.* **82**, 509.
5. Yphantis, D. A. (1964) *Biochemistry* **3**, 297.
6. Percival, E. (1970) in *The Carbohydrates* (Pigman, W. and Horton, D., ed.) Vol. IIB, pp. 537–568, Academic Press, New York.
7. White, R. C. and Barber, G. A. (1972) *Biochim. Biophys. Acta.* **264**, 117.
8. Hough, L. and Jones, J. K. N. (1962) in *Methods in Carbohydrate Chemistry* (Whistler, R. L. and Wolfrom, M. L., ed.) Vol. I, pp. 21–31 Academic Press, New York.
9. Roberts, K., Gurney-Smith, M. and Hills, G. J., (1972) *J. Ultrastructure Res.* **40**, 599.
10. Roberts, K. (1974) *Royal Soc. Lond., Phil. Trans.* **B268**, 129.
11. Lewin, R. A. (1956) *Can. J. Microbiol.* **2**, 665.
12. Horne, R. W., Davies, D. R., Norton, K. and Gurney-Smith, M. (1971) *Nature* **232**, 493.
13. Albersheim, P., Nevins, D. J., English, P. D. and Karr, A. (1967) *Carbohyd. Res.* **5**, 340.
14. Surzycki, S. (1971) in *Methods in Enzymology* (San Pietro, A., ed.) Vol. XXIII, Part A, pp. 67–73, Academic Press, New York.
15. Bernfeld, P. (1955) in *Methods in Enzymology* (Colowick, S. P. and Kaplan, N. O., ed.) Vol. I, pp. 149–158, Academic Press, New York.