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# THE CELL WALL OF *PERIDINIUM WESTII*, A NON CELLULOSIC GLUCAN

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SUMMARY

Cells of the dinoflagellate alga *Peridinium westii* were collected from water samples, taken from Lake Tiberias, during the period of algal blooms, and were purified by a procedure based on their phototaxis. Clean cell walls were isolated by mechanical disintegration of the purified whole cells, followed by differential centrifugation, and washing with detergents and organic solvents. The isolated walls were examined by physical, chemical and enzymatic techniques. The major component of the skeletal cell wall (about 95 % of its weight) was found to be a polymer of D-glucose which differed from cellulose in its X-ray diffraction pattern, solubility in Schweitzer reagent and cadoxen, and other physical and chemical properties. Partial degradation of cell wall glucan by acetolysis and acid hydrolysis afforded a series of cellodextrins ($n = 2$ to 4), as well as laminaribiose and laminaritriose, indicating that the glucose units are joined by $\beta$-(1 → 4)- and $\beta$-(1 → 3)-linkages. It has not been established whether the two types of linkages are present in the same polymer or in different polymeric molecules.

A pronounced degradation of the cell wall glucan by $\beta$-glucanases of snail digestive juice was achieved only when phosphoric acid-treated cell walls were used as a substrate. The relatively low rate of decomposition of the cell wall glucan, in comparison to other known glucans, under natural conditions, seems to be related to its closed structure which serves as a physical barrier for enzyme penetration.

## INTRODUCTION

Dinoflagellates frequently cause algal blooms, resulting in visible changes in turbidity or color in lakes, rivers and water reservoirs, as well as in seas[1]. This group of planktonic algae densely populates water reservoirs along the Israeli water supply lines. The cell walls of these algae represent a major part of the stable organic sediment of those water bodies[2]. Recent studies have shown that carbohydrates are the main constituent of algal cell walls; in addition, small quantities of proteins, lipids and ash have been detected in clean wall preparations[3,4]. Information on structural elements in the cell wall of planktonic algae is rather limited. Cellulose, mannan, xylan, alginic

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acid and fucinic acid have, however, been reported to be present in seaweeds[5–7].

One of the most common dinoflagellates found in lakes and water reservoirs in Israel is *Peridinium westii*. The cell wall of this organism has a firm skeletal layer, devoid of any mucilagenous material[8]. No information on the detailed composition and structure of this cell wall is presently available. Such information is needed for the understanding of the factors determining the high resistance of the cell wall of *P. westii* to biological degradation[2]. In this communication we describe studies on the chemical structure of this cell wall.

## MATERIALS AND METHODS

### *Cells and cell walls*

Repeated attempts to grow *P. westii* in the laboratory were not successful. The following procedure was therefore used to obtain a monoalgal culture of *P. westii*. *P. westii* cells were collected from Lake Tiberias (28.2.66) by a planktonic net made of No. 18 bolting silk[9]. The cell suspension obtained was concentrated by centrifugation in a Servall centrifuge (2000 rev./min, 10 min). The packed cells were suspended in tap water (1:20, v/v), and the *P. westii* cells were purified by a procedure based on their phototaxis[10]. The resulting suspension of *P. westii* cells was filtered through Whatman No. 1 filter paper, the precipitate resuspended in distilled water (1:10, v/v) and the cells disintegrated in a Manton–Gaulin homogenizer, model 15 M (Everett, Mass., U.S.A.) by recycling three times at 6000 lb./inch$^2$. The homogenate was centrifuged in a Servall centrifuge (5000 rev./min, 15 min), the supernatant was discarded and the pellet was resuspended in water (1:10, v/v) and centrifuged again at 5000 rev. per min for 15 min. The sediment consisted of cell walls, accompanied by some protoplasmic materials which were mostly chlorophyll pigments. These contaminants were removed by washing the precipitate with a series of solvents obtained by mixing equal volumes of Tween-80 in water (0.5 %, v/v) with ether, light petroleum (b.p. 40–60°), acetone and ethanol respectively. Three washings were performed with each solvent, using a ratio of 10 ml solvent to every ml of cells. The purified walls were then repeatedly washed with water until the sediment was white. The walls were suspended in water (1 ml of packed walls/100 ml water), and the suspension dialyzed against water in the cold for 72 h. The walls were collected by centrifugation and lyophilized. The final yield of walls was about 40 % of the dry weight of the cells (estimated by drying in vacuum over NaOH and $H_2SO_4$).

### *Carbohydrates*

Cellulose was a product of Balston, England (Whatman cellulose powder, standard grade). Pustulan, a $\beta$-(1 → 6)-glucan from the alga *Umbilicaria pustulata*[11], was kindly given by Dr. E. T. REESE, and paramylon, a $\beta$-(1 → 3)-glucan from *Euglena gracilis*[12], by Dr. K. MARECHAL. Laminarin (ex *Laminaria hyperborea*), a branched glucan with $\beta$-(1 → 3)- and $\beta$-(1 → 6)-linkages, was purchased from Koch–Light Laboratories. Starch was a product of Fluka, Switzerland (soluble starch A.R.). Laminaribiose and laminaritriose, $\beta$-(1 → 3)-oligosaccharides, were obtained as gifts from various laboratories. Cellodextrins were prepared by partial hydrolysis of cellulose[13]. All other saccharides used were from commercial sources and were of the highest purity available.

*Chemical reagents*

Schweitzer reagent, an ammoniacal copper hydroxide solution, was prepared according to the literature[14]. Cadoxen[15] was prepared as follows: 9 g of solid CdO were added at 0°, with vigorous stirring, to 1 l of a 28 % aq. solution (w/v) of fresh ethylenediamine. The mixture was allowed to stand overnight in a refrigerator, and the excess CdO was removed by centrifugation. To the supernatant (950 ml) a cooled mixture of 60 ml ethylenediamine, 155 ml of water and 14 g of NaOH was added.

*Enzymes*

Glucose oxidase (Glucostat) reagent was obtained from Worthington Biochemical Corp., Freehold, N.J. Cellulase was purchased from Nutritional Biochemical Corp., and crude β-glucosidase (emulsin) from Sigma Chemical Co. Snail enzyme, a digestive juice of *Helix pomatia* was a product of Koch–Light Laboratories.

*Physical techniques*

Electron micrographs were taken with a JEM-7 electron microscope. X-ray diffraction patterns of the powdered glucans were obtained with a Norelco Straumanis camera with a diameter of 57.3 mm, and cobalt radiation filtered through an iron foil. Exposures of approx. 1.5 h duration were made on Kodak No-screen medical X-ray film. The voltage used was 30 kV, and the current 10 mA. Infrared spectra were examined with a Perkin-Elmer infrared spectrometer as discs pressed in KBr.

*Analytical techniques*

Total sugars were estimated by the phenol-$H_2SO_4$ method[16], reducing sugars by the modified Park–Johnson method[17,44], and D-glucose was determined enzymatically by the glucose oxidase reagent. In these assays D-glucose (Pfanstiehl) was used as standard.

Nitrogen was estimated by the procedure of DUMAS[18] and phosphorus by the method of KING[19]. Ash was estimated by oxidizing the samples with fuming $HNO_3$ and then heating at 600° to constant weight. Amino acids were determined on the Beckman–Spinco amino acid analyzer, after samples in sealed ampoules had been hydrolyzed in 6 M HCl for 20 h at 100° (see ref. 20).

*Hydrolysis*

Cell walls (10 mg/ml) were hydrolyzed in 0.5 M $H_2SO_4$ at 100° for varying periods of time, up to 24 h. The hydrolysates were neutralized with solid $BaCO_3$; the $BaSO_4$ precipitate formed was removed by filtration, washed with hot water, and the combined filtrate and washings were lyophilized. Partial acid hydrolysis (10 mg cell wall per 1 ml of acid) was achieved in fuming HCl for 15 min at 4° (see ref. 21), or in 0.05 M $H_2SO_4$ for 4–24 h at 100°. For the estimation of rates of hydrolysis, samples of polysaccharides in 0.5 M $H_2SO_4$ (10 mg per 1 ml) were heated at 100° for varying periods of time, the hydrolysates were centrifuged and the supernatants analyzed for their content of total sugars (phenol test), and of D-glucose (glucose oxidase).

*Acetolysis*

Acetylation and deacetylation used for structural studies were performed according to the method described by GOLDSTEIN AND WHELAN[13]. Acetolysis, for the

determination of the total D-glucose content of the polymer, was according to SCHRAMM AND HESTRIN[22]. For the study of the oligosaccharides released during the reactions, the acetolyzed solutions were neutralized with $BaCO_3$, and desalted by Amberlite MB-3 (analytical reagent, Mallinckrodt).

*Oxidation studies*

Periodate oxidation (followed by spectrophotometry)[23], Smith degradation[24] and conversion of laminaribiose to arabinose by lead tetraacetate[25], were carried out according to the literature.

*Phosphoric acid treatment*

Phosphoric acid treatment of cell walls was carried out by suspending 10 mg of walls in 1 ml of $H_3PO_4$ (72 %, w/v) for 30 min at room temperature. The suspension was diluted with 9 ml water and the walls were collected by centrifugation. They were then washed with water in the centrifuge to neutrality, dialyzed against water for 72 h and lyophilized. No increase in the amount of reducing groups and in phosphorus was detected after this treatment.

*Enzymatic degradations*

Cell walls were degraded with snail enzyme. Snail enzyme diluted with sodium acetate buffer, 0.05 M at pH 5.8 (1:5, v/v), was added to 4 vol. of a suspension of cell walls (treated in phosphoric acid) in the same buffer (10 mg/ml). Following incubation for 60 min at 30°, the reaction was stopped by immersing the tubes in a boiling bath for 3 min, and the mixture analyzed for reducing sugars[17,44], and for D-glucose (glucose oxidase). An aliquot was also examined by paper chromatography in Solvent A (see below). Incubation for 8 h (under the conditions mentioned) was used for obtaining maximal amounts of oligosaccharides from the cell wall glucan with snail enzyme. Incubations with $\beta$-glucosidase were performed in sodium acetate buffer, 0.05 M (pH 5.0) at 30°, using a ratio of 1:2, by wt. enzyme/substrate.

*Paper chromatography*

For analytical purposes descending paper chromatograms were run on Whatman No. 1 paper, and for preparative purposes Whatman No. 3 was used with the following solvent systems: A, *n*-butanol–acetic acid–water (25:6:25, by vol.); B, *n*-propanol–ethylacetate–water (32:57:13, by vol.); C, ethylacetate–acetic acid–formic acid–water (90:15:5:2, by vol.); D, pyridine–ethylacetate–water (1:2:2, by vol.). Monosaccharides were detected on chromatograms by the use of $AgNO_3$ (ref. 26) or aniline phthalate[27]. Disaccharides and trisaccharides were located by $AgNO_3$ and by the diphenylamine–aniline spray[28].

*Column chromatography on charcoal–celite*[13]

Fractionation of saccharides obtained by partial chemical or enzymatic degradation of *P. westii* cell walls was carried out on a column (28 cm × 5.7 cm) of charcoal (Darco-G-60) and celite 535 (1:1, w/w). The column was activated by 6 M HCl, and equilibrated with distilled water. Elution was performed by a sequence of three linear water–ethanol gradients: (a) Mixing chamber, 1 l water; reservoir, 1 l ethanol 32 % (v/v); (b) mixing chamber, 500 ml ethanol 32 %; reservoir, 500 ml ethanol 64 %; (c) mixing chamber, 250 ml ethanol 64 %; reservoir, 250 ml ethanol 96 %. Fractions

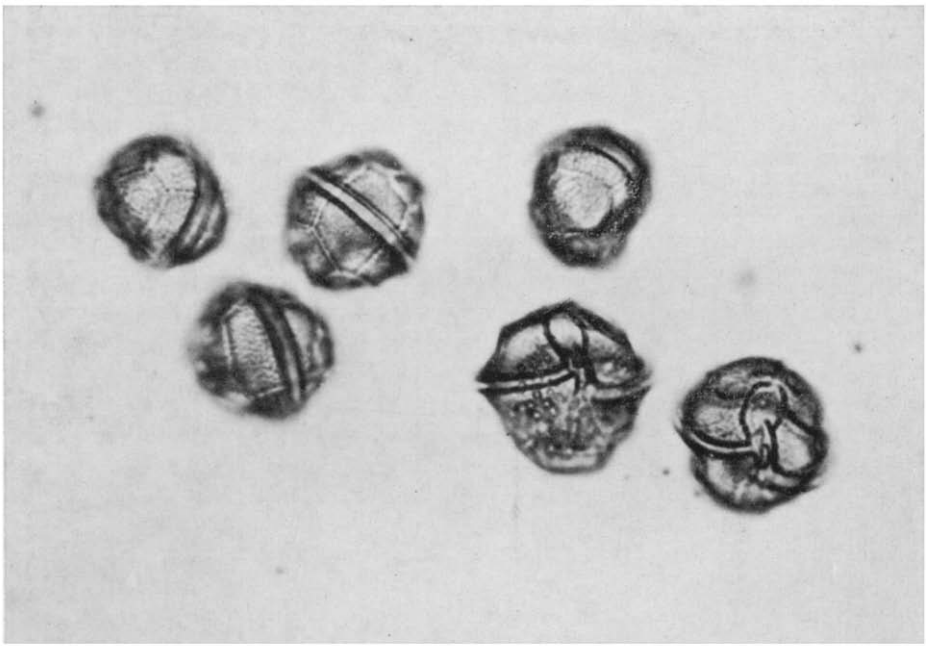

Fig. 1. *P. westii* cells (× 400), as seen under the phase contrast microscope.

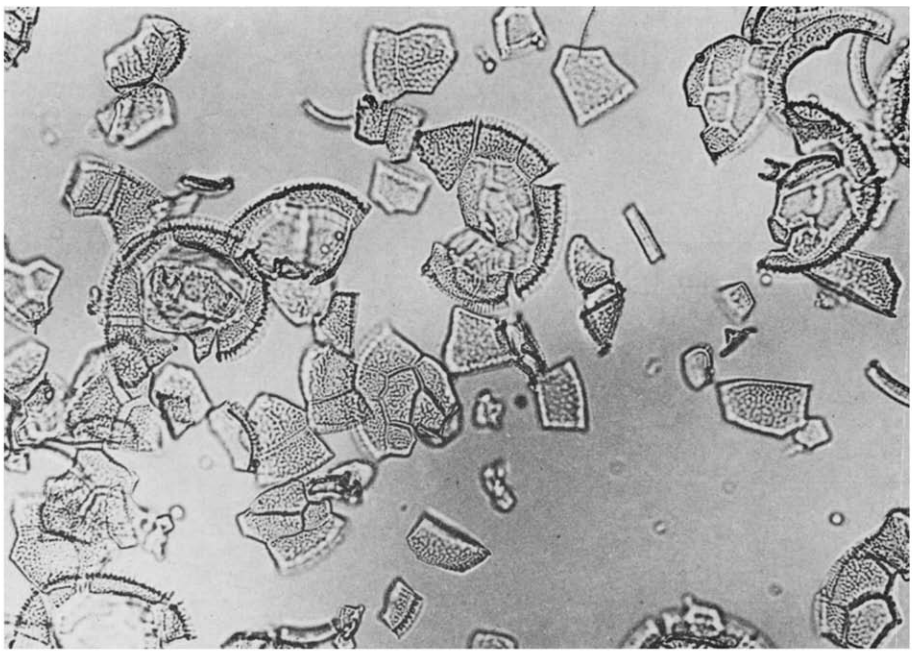

Fig. 2. *P. westii* cell walls (× 450), as seen under the phase contrast microscope.

of 10 ml were collected at a rate of 50 ml/h; 0.5-ml aliquots were taken for total sugars test (phenol method).

## RESULTS

Examination of the purified cells (Fig. 1) and cell walls (Fig. 2) under the phase contrast microscope, shows that the cells have the shape of spheres, where the walls are built of plates, singly or joined in groups. Such plate structures are in fact seen on the surface of the intact *P. westii* cells (Fig. 1). Electron micrographs of a cell section and of a cell wall section (Fig. 3), taken by Y. MESSER and Dr. Y. BEN-SHAUL[29], laboratory for Electron Microscopy, Tel Aviv University, show that the wall surrounds the cytoplasm, has a thickness of 2–3 $\mu$ and possesses some internal organization. Holes and canals are seen in the cell walls in all preparations examined, though we could not establish whether they are real structures, or artifacts from the method used in the preparation of sections for electron microscopy.

The cell walls do not dissolve in water, dilute acids or bases, cold strong phosphoric acid (72 %), nor in Schweitzer reagent (cupraammonium) or cadoxen[15]. The main constituents of the walls are carbohydrates (95% by the phenol test, see Table I). Only small quantities of nitrogen, phosphorus and ash were present (Table I). The inorganic fraction is composed mainly of silicon in the crystalline form of quartz and $\alpha$-cristobalite, as determined by X-ray diffraction of the ash, using copper radiation filtered through a nickel foil[30].

Analysis on the amino acid analyzer of an acid hydrolysate (6 M HCl, 100°, 20 h) of the walls revealed the presence of a wide range of amino acids, characteristic of proteins, in a total amount equivalent to 3 % of the weight of the walls. Examination of the wall powder by X-ray diffraction gave diagrams (3 in Fig. 4) which were different from those of cellulose or laminarin (1 and 2, respectively in Fig. 4). Infrared spectra exhibited an absorption at 11.2 $\mu$, characteristic for $\beta$-D-linkages. Treatment of the walls with acid under conditions commonly used for hydrolysis of neutral poly-

TABLE I

COMPOSITION OF *P. westii* CELL WALLS

| | *Dry walls (% of weight)* |
|---|---|
| Neutral sugars* | 95 |
| Protein | |
| Amino acids** | 3.0 |
| Nitrogen × 6.25 | 3.1 |
| Phosphorus | 0.22 |
| Ash (nitrated) | 0.40 |

* Phenol test carried out according to the method of DUBOIS *et al.*[16], with glucose as standard. Since the walls are insoluble in water, or in dilute acids or bases, the phenol tests were performed as follows: an accurately weighed amount of the walls which had been ground in a microblender was suspended in water (5–10 mg walls in 10 ml water). The fine suspension was mixed well, and aliquots (0.05 and 0.1 ml in triplicate) were taken for the phenol test and the color formed read with a Klett–Summerson photoelectric colorimeter, Filter No. 42. The results were reproducible within ± 3% (S.D.).

** Sum of amino acids found on the Beckman Spinco amino acid analyzer, in an acid hydrolysate (6 M HCl, 20 h at 100°) of the walls.

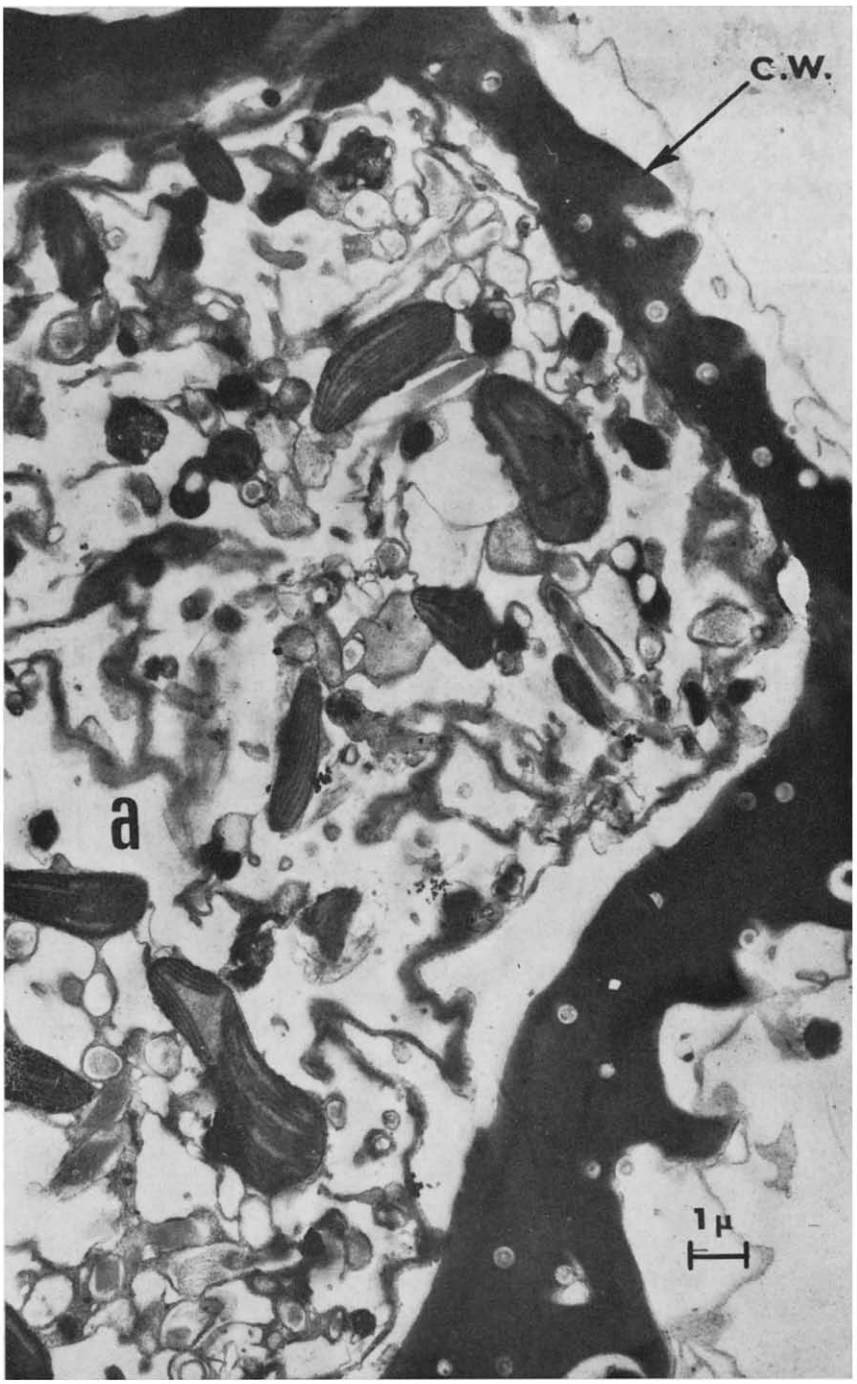


Fig. 3a. Electron micrograph of *P. westii* (× 7800). A cell section fixed in $OsO_4$ and glutaraldehyde, then stained by lead citrate. C.W. = cell wall.

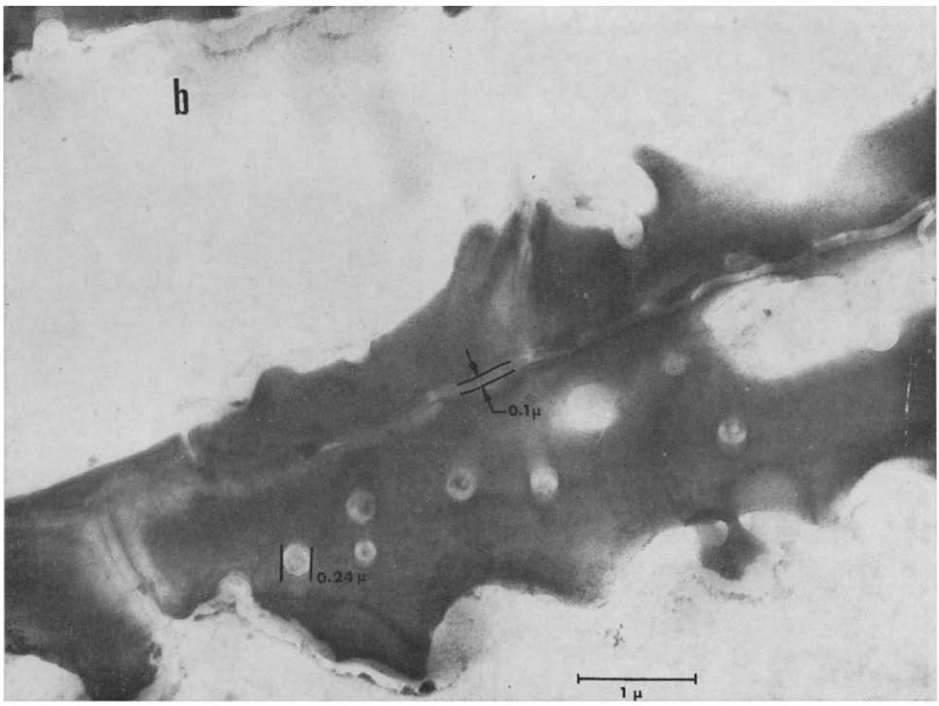


Fig. 3b. Electron micrograph of *P. westii* (× 17000). Cell wall section treated as in 3a. Note the canals and the holes. Prepared by MESSER and BEN-SHAUL, Tel Aviv University.

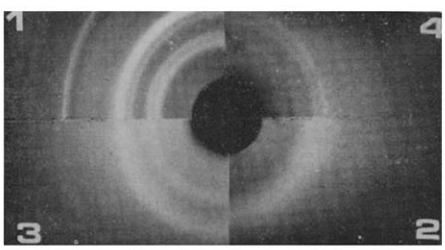


Fig. 4. X-ray diffraction diagrams of: Cellulose (1), laminarin (2), *P. westii* cell walls (3) and acid treated (0.5 M $H_2SO_4$, 6 h at 100°) (resistant fraction) *P. westii* cell walls (4).

saccharides ( 0.5 M $H_2SO_4$, 6 h, 100°), led only to their partial degradation. After this treatment 42 % of the walls were found in the soluble fraction, as reducing sugars, whereas 55 % remained insoluble. Paper-chromatographic examination of the soluble fraction showed that it was composed chiefly of glucose, with trace amounts of mannose, fucose and rhamnose. Small quantities of oligosaccharides were also present in the hydrolysate, since slow moving spots were seen on the chromatograms, using solvent systems A and B.

The acid resistant residue was washed with water to neutrality, and then dried (55 % of the weight of the intact walls). It remained undegraded on further treatment with acid (0.5 M $H_2SO_4$, 6 h, 100°), and was insoluble in Schweitzer reagent, but

dissolved in cadoxen. Its X-ray diffraction diagram (4 in Fig. 4) exhibited lines which were clearer than those found in the diagram of the untreated walls, but still differed from those of cellulose although to a lesser extent.

Both the intact walls and the acid resistant fraction, when subjected to complete acetolysis, gave D-glucose as the major product (Table II). Acetolysates of the latter fraction also contained trace amounts of mannose, fucose and rhamnose.

A comparison of the rates of hydrolysis of *P. westii* cell walls with those of different glucans is given in Fig. 5 and the periodate consumption curves in Fig. 6. Analysis of the glucose present in *P. westii* walls, the acid resistant fraction of the walls, cellulose and laminarin after hydrolysis of the Smith degradation products are

TABLE II

CARBOHYDRATES IN *P. westii* CELL WALLS

Hydrolysis was performed in 0.5 M $H_2SO_4$, 6 h, at 100°, and acetolysis according to SCHRAMM AND HESTRIN[22]. Sugars were estimated as D-glucose using the modified Park–Johnson method[17] or the Worthington glucose oxidase reagent.

| *Treatment* | *Analysis* | *Dry walls (% of weight)* |
|---|---|---|
| Acid hydrolysis* | Reducing sugars[17,44] | 42 |
| | Acid stable fraction | 55 |
| Acetolysis* | Reducing sugars[17,44] | 90 |
| | Glucose oxidase | 79 |

* Paper chromatography of both the hydrolysate and acetolysate revealed the presence of glucose as the major sugar, accompanied by trace amounts of mannose, fucose and rhamnose.

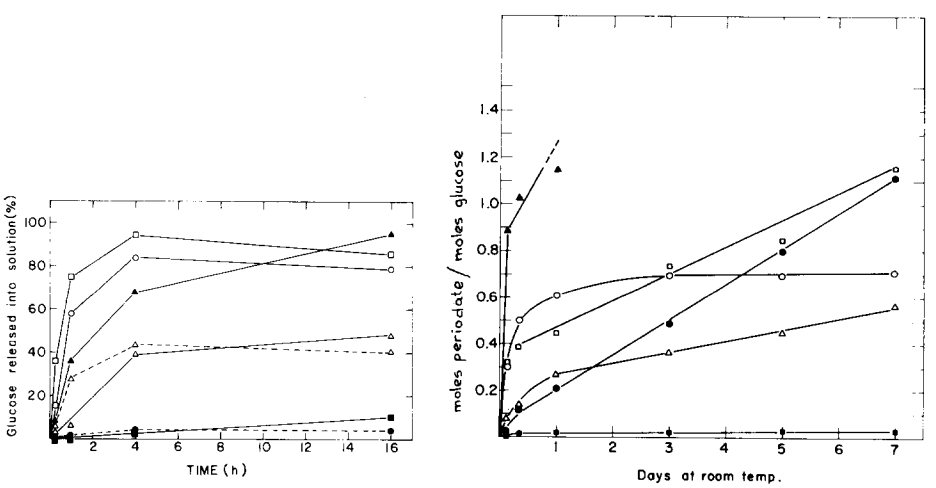


Fig. 5. The behavior of carbohydrates in acid hydrolysis (0.5 M $H_2SO_4$, at 100°), tested by glucose oxidase (———) and phenol (- - - - -). Starch (□), cellulose (●), laminarin (○), pustulan (▲), paramylon (■) and *P. westii* cell walls (△).

Fig. 6. Periodate uptake of glucans, followed at room temp. (23°). Cellulose (●), laminarin (○), pustulan (▲), paramylon (■), *P. westii* cell walls (△) and acid resistant fraction of *P. westii* cell walls (□).

given in Table III. It can be seen that, whereas in cellulose most of the glucose was destroyed by this treatment, yielding erythritol, in laminarin about 70% of the glucose remained intact. In *P. westii* walls, a significant proportion of the glucose residues remained intact after Smith degradation, whereas in the acid resistant fraction most of the glucose was destroyed. The oligosaccharides produced by the Smith degradation technique were hydrolysed separately by 6 M HCl at 100° for 30 min. Separation of the resulting saccharides by paper chromatography revealed the presence of glucose, erythritol and trace amounts of glycerol.

Additional information of the types of linkages found in *P. westii* cell walls, and their distribution, was obtained by partial degradation, both chemical and enzy-

TABLE III

SMITH DEGRADATION AND HYDROLYSIS OF GLUCANS*

| *Glucan* | *Hydrolysis conditions* | | | | |
|---|---|---|---|---|---|
| | *0.01 M HCl, 10 min at 100°* | | | | *6 M HCl, 30 min at 100* |
| | *Erythritol* | *Glycerol* | *Glucose (%)* | *Oligosaccharides*** | *Glucose (%)* |
| Cellulose | + | + | 5 | — | 3 |
| Laminarin | — | + | 0 | + | 70 |
| *P. westii* cell walls | + | + | 0 | + | 40 |
| Acid resistant fraction of *P. westii* cell walls | + | + | 3 | — | 3 |

* Glucans were oxidized by periodate and reduced by $NaBH_4$ under the conditions of the Smith degradation[24]. The reaction mixtures were then treated with acids as described in the table. The products of acid hydrolysis were examined by paper chromatography. In addition, glucose was measured by the glucostat reagent.

** The oligosaccharides present in the mixture of Smith degradation products were obtained by separating the mixture on Sephadex G-50, and were further hydrolysed.

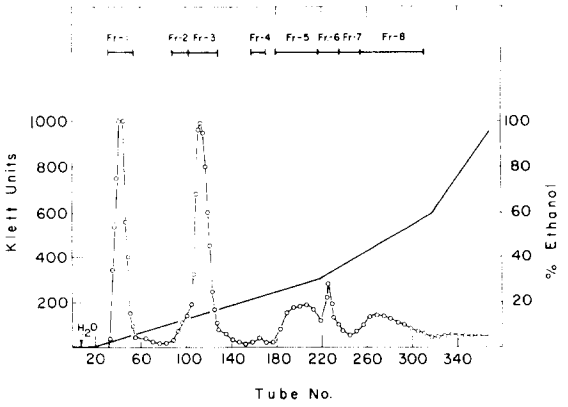


Fig. 7. Chromatography of an acetolysate[13] of *P. westii* cell wall glucan (1 g), on a charcoal–celite column (28 cm × 5.7 cm). The degraded glucan was dissolved in 10 ml of water and added to the column. 200 ml distilled water were passed before the elution with a linear gradient of increasing percentage of ethanol (for experimental details, see text). Fractions of 10 ml were collected at a rate of 50 ml/h. Aliquots (0.5 ml) were taken for total sugar tests, by the phenol method.

matic. Mild acetolysis afforded a mixture of oligosaccharides which was fractionated on a charcoal–celite column (Fig. 7). Fractions obtained from the column were examined by paper chromatography. Fraction 1 was composed mainly of glucose, and was not further investigated. Fractions 2, 3 and 4 contained mainly disaccharides and Fractions 5–8 mainly tri- and tetrasaccharides. Fractions 2–8 were further purified by preparative paper chromatography in Solvent A, yielding six oligosaccharides. These oligosaccharides were identified by their rates of migration on paper, using four different solvent systems, and by chemical and enzymatic tests, as laminaribiose (I), laminaritriose (III), laminaritetraose (V), cellobiose (II), cellotriose (IV) and cellotetraose (VI) (Table IV). $R_{glucose}$ values, composition and yields of the six oligosaccharides are given in Table IV. Both series of homologous oligosaccharides ($\beta$-(1 → 3) and $\beta$-(1 → 4)) gave straight lines when $2 - \log R_{glucose}/(1-R_{glucose})$, ($R_{glucose}$ in solvent A) were plotted against the number of glucose units[31]. Acid hydrolysis (0.5 M $H_2SO_4$, 30 min, at 100°) of isolated disaccharides (I and II), trisaccharides (III and IV) and tetrasaccharides (V and VI), resulted in the release of about two, three and four reducing equivalents, expressed as glucose, per equivalent of saccharide respectively (as measured by the modified Park–Johnson test, see Table IV). The $\beta$-configuration of each of the six oligosaccharides was proven by their almost complete degradation to glucose, upon incubation for 24 h with $\beta$-glucosidase. Maltose was not degraded under identical conditions. Short incubations of 10–30 min of the $\beta$-(1 → 4)-oligosaccharides with $\beta$-glucosidase yielded cellobiose and glucose from oligosaccharide IV, and cellotriose, cellobiose and glucose from oligosaccharide VI, as detected on paper chromatograms in Solvent A. Similar experiments were

TABLE IV

OLIGOSACCHARIDE COMPONENTS OF *P. westii* CELL WALLS OBTAINED AFTER PARTIAL ACETOLYSIS

Acetolysis was performed according to GOLDSTEIN AND WHELAN[13]. 1 g of *P. westii* cell walls was subjected to the acetolysis. Oligosaccharides were chromatographed on a charcoal–celite column (Fig. 7), followed by preparative paper chromatography.

| | *Yield (mg)* | *Ratios of reducing power expressed as moles of glucose before and after hydrolysis** | $R_{glucose}$ *in solvent system* | | | |
|---|---|---|---|---|---|---|
| | | | *A* | *B* | *C* | *D* |
| Oligosaccharide I | 9 | 1 : 1.90 | 0.68 | 0.62 | 0.54 | 0.98 |
| Laminaribiose | | | 0.69 | 0.64 | 0.55 | 0.98 |
| Oligosaccharide II | 23 | 1 : 1.95 | 0.56 | 0.40 | 0.45 | 0.84 |
| Cellobiose | | | 0.56 | 0.42 | 0.45 | 0.84 |
| Oligosaccharide III | 7 | 1 : 2.9 | 0.40 | 0.24 | 0.20 | 0.76 |
| Laminaritriose | | | 0.40 | 0.24 | 0.20 | 0.76 |
| Oligosaccharide IV | 16 | 1 : 3.0 | 0.28 | 0.17 | 0.09 | 0.62 |
| Cellotriose | | | 0.28 | 0.18 | 0.10 | 0.62 |
| Oligosaccharide V | 4 | 1 : 3.8 | 0.20 | 0.11 | 0.03 | 0.54 |
| Oligosaccharide VI | 19 | 1 : 3.7 | 0.10 | 0.06 | 0.01 | 0.32 |
| Cellotetraose | | | 0.10 | 0.06 | 0.01 | 0.32 |

* Solutions of the different oligosaccharides were assayed for their total glucose content by the phenol method[16] and for reducing groups by the modified Park–Johnson test[17]. An aliquot was hydrolyzed (0.5 M $H_2SO_4$, 30 min at 100°), and estimated for reducing groups.

performed on oligosaccharides III and IV, yielding mostly glucose, and only traces of dimer and trimer. Mild treatment of the $\beta$-(1 $\rightarrow$ 3)-oligomers with NaOH (0.01 M in the cold overnight), transformed all of the oligomers to D-glucose, while similar treatment of the $\beta$-(1 $\rightarrow$ 4)-oligomers yielded only trace amounts of glucose.

Oxidation of oligosaccharide I (laminaribiose) by lead tetraacetate[25], followed by acid hydrolysis of the oxidized mixture, yielded arabinose and glucose, as shown by paper-chromatographic analysis. Similar treatment of oligosaccharide II (cellobiose) yielded glucose and erythrose, but not arabinose.

*Enzymatic experiments*

Incubation of purified cell walls with cellulase and snail enzyme, separately and combined, did not cause an increase in reducing groups, or a release of glucose residues. However, both phosphoric acid-treated cell walls, and the acid resistant fraction of the walls, obtained after hydrolysis in 0.5 M $H_2SO_4$ for 6 h at 100°, were partially degraded by the two enzyme preparations. Phosphoric acid-treated walls were a better substrate than the acid resistant fraction. Following incubation for 60 min with the snail enzyme, 15 % by wt. of the phosphoric acid-treated walls was converted to a soluble form, as tested by the modified Park–Johnson method. The insoluble residue, recovered by washing and drying, accounted for about 80 % of the weight of the walls. This residue was submitted to four successive incubations with snail enzyme. After each period of incubation (12 h) the cell wall residue was removed by centrifugation, washed, fresh enzyme was added, and the mixture was incubated again. Starting with 100 mg of cell walls, a residue of 6 mg was obtained, indicating that 94 % by wt. of the walls were solubilized.

## DISCUSSION

In this paper we describe a method for isolating *P. westii* cells from water samples containing a wide range of planktonic algae. By this method large quantities of purified cells could be readily obtained, although the problem of growing *P. westii* in the laboratory has not yet been overcome. Cell walls were isolated from the cells by mechanical disintegration followed by repeated washings, yielding a homogeneous preparation, which served for the present study. Similar methods are used for preparation of cell walls of other microorganisms like bacteria and fungi[7]. The cell walls possess the shape of plates (Fig. 2) and their morphology is that excepted from examination of whole cells (Fig. 1). Electron micrographs of sections of *P. westii* cells and cell walls show that the wall is a compact layer 2–3 $\mu$ in width, and is highly organized. Cell walls of unicellular algae are generally believed to consist of cellulose. The results described in the present report show that cell walls of the dinoflagellate alga *P. westii* are made up chiefly of a glucose polymer, or a mixture of glucose polymers, which differ in many of their physical and chemical properties from cellulose. The isolated cell walls exhibited X-ray diffraction patterns different from that of cellulose (Fig. 4), and were not soluble in Schweitzer reagent or in cadoxen. A $\beta$-configuration of the glycosidic bonds in the polymer was suggested by the infrared absorbance at 11.2 $\mu$, and by the complete degradation by $\beta$-glucosidase of the oligosaccharides isolated from partially degraded walls. Since glucose was the main sugar present after complete hydrolysis and acetolysis of the walls, tests were performed to

compare *P. westii* cell walls with a series of β-glucans, such as cellulose, laminarin, paramylon and pustulan. The results of acid hydrolysis (Fig. 5) and periodate oxidation experiments (Fig. 6), suggested the presence of two kinds of glycosidic linkages, β-(1→3) and β-(1→4), as the main types of linkage in the glucan or glucans of the wall. The high uptake of periodate observed with laminarin (Fig. 6), may seem surprising. It is known, however, that laminarin contains variable proportions of β-(1→3)- and β-(1→6)-linkages, and is branched to different extents[32]. Uptakes as high as 0.58 mole periodate per mole of anhydroglucose have been observed upon periodate oxidation of laminarin[33]. WOLFROM, THOMPSON AND TIMBERLAKE[34] have shown that the order of increasing stability of glycosidic linkages to acid hydrolysis in glucose disaccharides is: (α-(1→2), α-(1→4)) → (β-(1→2), β-(1→3)) → (β-(1→6), β-(1→4)) → (α-(1→6)). It seems, however, that in polysaccharides other factors in addition to the type of glycosidic bond may influence stability. Paramylon, and laminarin, both β-(1→3)-glucans (the latter with β-(1→6)-linkages as well), behaved differently upon acid hydrolysis, hinting at the possible effects of length, linearity and intermolecular bonds.

The presence of β-(1→4)- and β-(1→3)-linkages in the glucan of *P. westii* cell walls is supported by the results of degradation studies. After Smith degradation of the cell walls, 40 % of the glucose remained intact (Table III), indicating the presence of (1→3)-linkages; on the other hand, concomitant formation of erythritol is evidence for the existence of (1→4)-linkages. Small amounts of glycerol were also found after Smith degradation. Further and more conclusive evidence for the presence of the two types of linkages in *P. westii* cell wall glucan or glucans is based on the isolation and characterization of a series of β-(1→3)- and β-(1→4)-glucose oligosaccharides from partial acetolysates of the walls (Table IV). These oligosaccharides contained the expected amounts of glucose, they were degraded by β-glucosidase, and migrated on paper in several solvent systems, at rates identical to those of authentic β-(1→3)- and β-(1→4)-oligomers. In this connection it is pertinent to note that trisaccharides with mixed linkages were found by CHEN AND LUCHSINGER[35] to have different migration rates on paper, from those of the corresponding homologous oligosaccharides (cellotriose and laminaritriose). Oligosaccharides identical with those found in the acetolysate were also isolated from partial acid hydrolysates (0.05 M $H_2SO_4$, 24 h, 100°), and from incubation mixtures with snail enzyme (6 h at 30°) of phosphoric acid-treated walls.

The yields of the β-(1→3)-oligomers from the partial degradation mixtures were much lower than those of the corresponding β-(1→4)-oligomers. In the case of the chemical degradations (partial acetolysis and partial hydrolysis), this is most likely due to the higher lability of β-(1→3)- as compared to β-(1→4)-linkages.

The acid resistant fraction of the cell wall glucan resembles cellulose much more closely than the intact cell wall. While there are still differences in the X-ray diffraction pattern and solubility behavior in Schweitzer reagent, the acid resistant fraction shows a number of properties similar to those of cellulose (solubility in cadoxen, resistance to acid hydrolysis, relatively high uptake of periodate and concomitant destruction of glucose). Most likely an enrichment of β-(1→4)-linkages took place during hydrolysis. Upon Smith degradation of the acid resistant fraction, the major polyalcohol detected was erythritol, but the proportion of glycerol was higher than that formed upon Smith degradation of the intact walls. This indicates that the carbohydrate chain of the resistant fraction is shorter than that of the wall polymer.

From the percentage of glucose unaffected by periodate on one hand (Fig. 6 and Table III), and from the proportion of the acid resistant fraction (Fig. 5) on the other hand, it may be presumed that in the intact wall the two types of linkages are in a ratio of approx. 1:1. Our present data do not enable us to conclude whether the two types of linkages are present in the same macromolecule, or if the cell wall is a mixture of two or more glucans, differing in the types of linkages and in their distribution.

Natural mixed $\beta$-(1 → 3)- and $\beta$-(1 → 4)-glucans from lichenin, oat and barley are highly soluble in water and are degraded easily by glucanases[37]. It has been suggested (see ref. 36, p. 397) that the presence of two kinds of glycosidic linkages in one chain disrupts the linear orientation of a molecule, which in turn increases its solubility in water. FLEMING AND MANNERS[38] showed that properties of mixed glucans ($\beta$-(1 → 3), $\beta$-(1 → 4)) are determined mainly by the sequence of the linkages, and are less dependent on the total ratio in the polymer. Thus, a possible explanation for the odd properties of *P. westii* cell wall glucan(s) lies in a special arrangement of the blocks of the two types of linkages, in one polymer or in a tight arrangement of the different polymers, and to a lesser degree in their ratio. Strong intermolecular bridges, presumably hydrogen bonds, may play a key role.

*P. westii* cell walls became susceptible to glucanases from *Helix pomatia* only after a pretreatment using phosphoric acid. A similar treatment of cellulose with acids, as well as grinding, is known to yield a swollen material susceptible to enzymatic digestion[39,40]. Structural materials are known to be packed in closed forms[41]. Such a closed structure may limit the penetration of enzymes. Phosphate treatment (or time in natural conditions) may break the intermolecular secondary valence attachments, and allow the opening of the structure.

In spite of the immense variety of algal forms, their cell walls seem to have a common structural pattern which involves at most three distinct layers. In some of the algal classes only the inner compact layer is present, as in the Dinophyceae, to which *P. westii* belongs. It was believed that this layer usually consists primarily of cellulose, the prime structural polysaccharide. The present findings show that *P. westii* has a cell wall layer consisting of another structural glucan(s) which differ significantly from cellulose. A similar, though soluble, glucan is synthesized by the alga *Monodus subterraneus*[42].

Information on the chemical structure of algal cell walls is important for the management of water reservoirs. Algal cell wall residues can accumulate and serve as a constant source of organic matter[43]. The elucidation of the factors affecting their biodegradation is an important step towards the understanding of the biology of water bodies.

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