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The Comparative Aspects of Cell Wall Chemistry in the Green Algae (*Chlorophyta*)*

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Summary. The origin of a cell wall was an event of fundamental importance in the evolution of plants. In the green algae, cell walls apparently had independent origins in at least three lines of evolution. In this paper, the components of the cell wall were determined and compared in four filamentous green algae representing the charophycean, chlorophycean and ulvacean evolutionary lines. The walls of all four have hydroxyproline-containing proteins which separate into five or six bands upon SDS gel electrophoresis. Variation does exist, with the charophyte possessing fast moving electrophoretic bands and high hydroxyproline content, the chlorophytes having intermediate movement of bands and lower hydroxyproline content, and the ulvacean representative possessing slow moving bands and a very low, if not questionable, hydroxyproline and saccharide content. Qualitative and quantitative estimates of wall proteins and sugars have been determined and compared. A hypothetical scheme of cell wall evolution based on these data, those of previous analyses, and recent phylogenetic schemes is presented. Although sound conclusions cannot be made until more information is available, the scheme might help to emphasize the areas most in need of additional research.

Key words: Green algae – Phylogeny – Cell walls – Glycoproteins

Introduction

The origin of a cell wall is one of the most distinctive and important occurrences in the evolution of algae and higher plants (Mattox and Stewart, 1977; Stewart and Mattox, 1978; Swain, 1972). The cell wall not only provided early plant cells with an osmotic control and a rigid structure which allowed the elaboration of coherent multicellular forms, but also altered some cytoplasmic processes secondarily. Examples of such alterations might be variations in structural details of mitosis and cytokinesis in some algal groups. There is evidence that extracellular scales fused to form a theca or wall

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in certain green flagellates ancestral to the Chlorophyceae (Stewart and Mattox, 1978; Mattox and Stewart, 1977; Manton and Parke, 1965), and from that juncture, typical chlorophycean cell walls developed. The rigidity of the wall and the consequent inability of the protoplast to elongate during mitosis was probably the major factor in the alteration of mitosis and cytokinesis characteristic of the Chlorophyceae (Mattox and Stewart, 1977).

It is now apparent that land plants (embryophytes) had their origin with the Charophyceae (*sensu lato*, including the orders, Klebsormidiales, Zygnematales, Trentepohliales, Colechaetales and Charales) while the Ulvaceae and Chlorophyceae diverged from that stock before the origin of colonial or filamentous forms (Stewart and Mattox, 1975; Mattox and Stewart, 1977). There is no evidence that the cell walls of the Ulvaceae and Charophyceae evolved from the fusion of scales as in the flagellated ancestors of the Chlorophyceae. The zoospores of the Ulvaceae and Charophyceae often have body scales, suggesting that scales have not been altered in the evolution of the cell wall. Furthermore, the presence of scales in the zoospores demonstrates that cell walls evolved in nonflagellated, coccoid or dormant stages rather than in flagellated forms (Mattox and Stewart, 1977). The ontogenetic development of cell walls under the scales of germlings growing from scaly zoospores (as in *Pseudendoclonium*, Mattox and Stewart, 1973) might, in fact, indicate the mode and site of evolution of the cell wall in the Ulvaceae and Charophyceae.

It is known that the outer covering of plants consists of matrical and fibrillar polysaccharides with a characteristic glycoprotein, sometimes termed extensin, containing varying amounts of arabino-hydroxyproline residues (Preston, 1975; Miller et al., 1972; Lamporte et al., 1971; Albersheim, 1976). Those substances have been investigated in many plants, but relatively little has been done with green algae (Miller et al., 1972; Gooday, 1971; Lewin, 1958; Kuo-shii and Barber, 1975; Catt et al., 1976; Roberts, 1974). It is the purpose of this paper to examine the walls of four lower green algae, representing three apparent lines of green algal evolution, the Charophyceae, Chlorophyceae, and Ulvaceae, to determine whether ultrastructural variations of the protoplast are paralleled by chemical variations in the cell wall.

Materials and Methods

The algae used in this study were *Klebsormidium flaccidum* (UTEX LB 2017), representing the Charophyceae, *Ulothrix belkae* (UTEX 1179) and *Pleurastrum terrestre* (UTEX 333) representing the Chlorophyceae, and *Pseudendoclonium basiliense* (UTEX 1913), representing the Ulvaceae. Mass quantities of the organisms were grown in multiple, one liter quantities of Bold Basal Medium in two liter, Fernbach, Lo-Form flasks, that were bubbled constantly with sterile air. Cultures were harvested at 14 days of growth in 12/12 light period at 20°C. Cell walls were isolated in one of two ways. In one process, the algae were collected by suction filtration with glass fiber filters, scraped off and placed in a Ten Broeck homogenizer, and macerated in cold 0.01 M sodium phosphate buffer (pH 7.0). This process was repeated several times until filtering yielded a white residue, and light microscopic analysis showed that only broken cell walls remained in the maceration. In certain cases, a liter of harvested algae was oven-dried and weighed, followed by the above procedures to determine the weight of the wall

in relation to total cellular weight. A second technique used in isolating walls is a variation of ethanol extraction-precipitation, in which the residue of walls, homogenized and filtered, was subsequently plunged into boiling ethanol for ten second intervals, after which they were filtered with cold ethanol rinses. Neither of the two techniques proved to be superior in analyses of the results. Cell walls were then dried in sterile air and stored at 20°C.

Histological analyses for the presence of wall constituents, such as sporopollenin (Atkinson et al., 1972), lignin (Jensen, 1962), and chitin (Jensen, 1962); Herth et al., 1977), were performed on both isolated cell walls and intact plants.

Dried isolated walls were separated into the various hot water-soluble, base (4N KOH)-soluble, and acid (67% H_2SO_4)-soluble fractions by established techniques (Northcote et al., 1958). Protein determination was carried out by the method of Lowry et al. (1951). Qualitative examination of the amino acids in the walls was performed as follows: 50–100 mg of wall material was hydrolyzed in 6N HCl for 6 h at 105°C. After this time, the extract was cooled and neutralized with $BaCO_3$ and dried in vacuo over KOH. Repeated drying and rehydration took place to purify the extract. Qualitative identification of the amino acids was performed by two-dimensional paper chromatography as described previously (Spiro, 1966). Sugars from the above hydrolysates were chromatographed by a similar method (Spiro, 1966). Identification sprays were as follows: Ninhydrin (0.5% in acetone) for amino acids and aniline hydrogen phthallate (1% in a 10% phosphoric acid solution) for sugars. Similarly, modified Dische spectra (Keleti and Lederer, 1974) were taken for sugar identification, with scanning from 740 to 380 nm upon a Perkin-Elmer UV-Visible spectrophotometer (Model 124).

The quantitative identification of specific sugars and amino acids in the walls was performed as follows: hydroxyproline was measured by the modified technique of Leach (1960); glucosamine, uronic acid, rhamnose, mannose and total pentose were determined by the technique of selective hydrolyses and colorimetry (Keleti and Lederer, 1974; Spiro, 1966). Total cellulose in the walls was quantified by the Updegraff (1969) method of detection.

The separation of protein from the wall was performed basically by the techniques of Roberts (1974) and Horne et al. (1971). 100 mg of cell wall material were digested in a 1% sodium dodecyl sulfate (SDS) solution containing 1% β-mercaptoethanol and a 0.01 M sodium phosphate buffer (pH 7.0) at 37°C for either 6, 12 or 24 h. Time variations altered the results insignificantly. The digested samples were then dialyzed overnight in a 0.01 M sodium phosphate (pH 7.0) buffer. Disc electrophoresis was performed as follows: Gels were molded 10 cm long without stacking gels. The gels contained 5% acrylamide, 0.01 M sodium phosphate buffer, 0.2% SDS, 45 μl of tetramethyl ethylenediamine per 30 ml of gel solution and 15 mg/ml of ammonium persulfate. Protein sample digest was centrifuged on a clinical centrifuge to remove any remaining debris and 100 μl of supernatant was applied to each gel. Each sample also contained 0.001% bromophenol blue (marker dye), 1% β-mercaptoethanol and 0.1% SDS. A continuous sodium phosphate (0.01 M) buffer system was utilized in the separation and a current of 8 ma/tube was applied until the marker dye just reached the bottom of the tubes. The gels were then removed from the tubes, quickly washed in 0.01 M sodium phosphate buffer (pH 7.0) and stained in the following ways: Periodic Acid Schiff (PAS) for glycoprotein staining by the method of Keyser (1964), and

Coomassie Blue for protein by the method of Horne et al. (1971). R_E values were obtained by measuring the gels with an analytical comparator over a fluorescent light box.

Results and Discussion

1. Proteins and Amino Acids

Cell wall protein from all test organisms was isolated, extracted, and analyzed from 6N HCl hydrolysates (Gooday, 1971; Spiro, 1966) and 1% SDS digestion (Horne et al., 1971) from purified walls, both non-treated walls and remnants of water and base hydrolysis. By paper chromatography and electrophoresis, followed by staining with Coomassie Blue and PAS, the 'protein' from *Klebsormidium flaccidum* (Charophyceae), *Ulothrix belkae* (Chlorophyceae), and *Pleurastrum terrestre* (Chlorophyceae) was found to be glycoprotein containing arabinose, xylose, galactose and eighteen amino acids (see figure for listing). *Pseudendoclonium basiliense* (Ulvaceae) possessed wall protein, but no determinable glycan region could be detected by subsequent chromatographic analyses.

Upon electrophoresis, the proteins in all algae were differentiated into five or six bands (see Table 1), *K. flaccidum* had six relatively fast moving bands; *U. belkae* and *P. terrestre* had five (or six) bands of medium R_E values; and *P. basiliense* had five bands of slow moving character. Bands at 0.44–0.46 and 0.51–0.53 displayed strong staining properties with other bands showing weaker intensity in both PAS and Coomassie Blue staining. Molecular weight observations were made by comparison with

Table 1. Partial characterization of wall protein. Key: * = Amino acids found: ala, asp, asn, glu, gln, gly, val, tyr, pro, hyp, lys, his, leu, ile, phe, ser, thr, cys. All = all bands gave a positive test upon staining. None = all bands gave a negative test upon staining

	Alga			
	K. flaccidum	*U. belkae*	*P. terrestre*	*P. basiliense*
Constituent				
Total protein	22.6	24.0	37.3	20.0
Hydroxyproline				
Wall (%)	1.8	0.6	0.9	0.2
Hydroxyproline				
Protein (%)	7.6	2.4	2.5	0.6
P.C. identification of				
amino acids	*	*	*	*
PAS stain of gel bands	all	all	all	none
Coomassie blue stain of				
gel bands	all	all	all	all
R_E values of protein upon	0.40	0.44	0.43	0.38
SDS gel electrophoresis	0.46	0.47	0.47	0.40
	0.51	0.52	0.53	0.41
	0.55	0.58	0.58	0.43
	0.58	0.62	0.61	0.55
	0.66			

bovine albumen, catalase, egg white lysozyme and pectinase proteins. Hydroxyproline was a characteristic amino acid of cell wall proteins, and was found in varying amounts in all test algae. *K. flaccidum* had the most, with 1.8% of the wall (or 7.6% of the protein) being hydroxyproline; *U. belkae* and *P. terrestre* possessed comparable levels of 0.6 and 0.9% of the walls respectively (or 2.4 and 2.5% of the wall protein respectively); and *P. basiliense* had low levels of 0.2% of the wall (or 0.6% of the protein).

These results indicate that four widely divergent green algae possess hydroxyproline protein in their walls. *P. terrestre* and *U. belkae* have a glycoprotein with hydroxyproline levels much less than *Chlamydomonas* (Lamporte and Miller, 1974; Roberts, 1974) and *Volvox* (Lamporte and Miller, 1974), all of which are chlorophycean algae. *P. basiliense* possessed a distinguishable wall protein but no discernable glycan region. It is also important to note that *Chara* and *Staurastrum* have been reported (Gotelli and Cleland, 1968) to have no hydroxyproline glycoprotein, but the charophycean alga, *K. flaccidum*, possesses a definite hydroxyproline protein.

2. *Polysaccharides*

In regard to water soluble polysaccharides (Table 2) two types amidst the four test organisms were determined. *K. flaccidum*, with an extensive mucilaginous sheath around the filament, possessed a high amount of water soluble polysaccharides (38%). *U. belkae* had a similar amount (39%), while *P. terrestre* and *P. basiliense* have lower amounts (31.5% and 30% respectively). It should be noted that the two organisms which possess the highest amounts of water soluble polysaccharides are primarily inhabitants of supralittoral freshwater streams. The existence of a large sheath of water soluble saccharides may be a protection against freezing or desiccation. The two algae with the lower amounts of wall saccharide soluble in water are terrestrial, usually found in shaded conditions.

Table 2. Percentages of various cell wall constituents including complex polymers and key saccharides

	Alga			
Constituent	*K. flaccidum*	*U. belkae*	*P. terrestre*	*P. basiliense*
Water soluble polysaccharides	38.0	39.0	31.5	30.0
Base soluble polysaccharides	29.0	23.3	18.2	25.1
Cellulose	6.7	13.3	12.4	16.6
Total protein	22.6	24.0	37.3	20.0
Additive amount toal wall %	96.3	99.5	99.4	91.7
Wall weight / Cell weight	36.7	25.0	41.0	12.8
Total pentose	10.0	1.8	1.4	2.2
Total rhamnose	3.0	2.3	2.5	6.0
% rhamnose in water soluble material	7.9	5.8	7.9	20.0
% glycuronic acid in the wall	11.6	30.0	20.5	10.5
% glycuronic acid in water soluble material	30.5	76.8	65.0	35.0
Lignin	–	–	–	–
Sporopollenin	–	–	–	–

Table 3. Paper chromatographic analysis of polysaccharide fractions in wall. Key: glc – glucose, gal – galactose, glcu – glycuronic acid, galu – galacturonic acid, xyl – xylose, ara – arabinose, rhm – rhamnose

Constituent	Alga			
	K. flaccidum	*U. belkae*	*P. terrestre*	*P. basiliense*
P.C. identification of water soluble material	glc, glcu, gal, galu, xyl, rhm,	glcu, galu, glc, xyl, rhm	glcu, glc, gal, galu, xyl, rhm	glc, glcu, gal, galu, xyl, rhm
P.C. identification of base soluble material	glc, xyl, ara	glc, xyl, ara, glcu	glc, xyl, ara	glc, xyl, ara
P.C. identification of 67% H_2SO_4 material	glc	glc	glc	glc

K. flaccidum's water soluble polysaccharides, upon chromatographic analysis, showed the presence of glycuronic acid, galacturonic acid, glucose, galactose, xylose and rhamnose (Table 3). Upon spectrophotometric examination, rhamnose was deemed to represent 7.9% of the water soluble layer. It is possibly significant that the charophycean, *K. flaccidum*, related to the ancestry of land plants, has amounts of rhamnose comparable to that found in higher plants (Albersheim, 1976). *U. belkae*'s wall possessed a similar sheath and has a 77% organic acid content and a 5.8% rhamnose content in the water soluble layer. These percentages are atypical in regard to other chlorophycean algae, because *Chlorella* has been reported (Kuo-shii and Barber, 1975) to have rhamnose constituting 47% of the water soluble layer, and little or no uronic acid. *P. terrestre* had 65% acid residues and 7.9% rhamnose, more closely similar to *U. belkae* than *Chlorella*. *P. basiliense* has a water soluble saccharide content of 30% uronic acid and 20% rhamnose, very similar to the *Ulva lactuca*-type water soluble saccharide reported previously (Percival and McDowell, 1967). Sugars found in *U. belkae, P. terrestre*, and *P. basiliense* were similar to those found in *K. flaccidum*, except that galacturonic acid was not found in *U. belkae*.

The base soluble wall fractions or 'hemicellulose' separated the algae into two groups. *K. flaccidum* belonged to one group which corresponded to high levels of hemicellulose (29–32%), while the other three algae had correspondingly lower amounts from 25.1 to 18.2%. All base soluble fractions displayed glucose, xylose and arabinose upon chromatographic analyses. It is known (Albersheim, 1976) that the hemicellulose consists of a linear cellulose-like glucan with xylan side chains or other sugar side chains, preventing close lateral associations and hence restricting microfibril development and lowering the wall's crystallinity. Variations of this hemicellulose (Preston, 1975) occur in higher plants, and studies with algae also show this. The high amount of hemicellulose in *K. flaccidum* corresponds to a lower level of cellulose and hence a lesser degree of crystallinity.

The amount of cellulose produced by the four test organisms also varied. *P. terrestre* and *U. belleae* contained comparable amounts of 12.4 and 13.3% respectively. *P. basiliense* contained 16.6% cellulose, similar to *Ulva* (Percival and McDowell, 1967), and *K. flaccidum*, an organism on the land plant line of evolution, possessed a low amount of 6.7%. The low amount pertaining to *K. flaccidum*, is somewhat surprising

and may indicate that this organism is a side branch member from the main line of green algae leading to the higher plants, which have a higher cellulose content. The algae in the cellulose test represent three separate types corresponding to the three lines of green algal evolution. Their prasinophyte ancestors have not yet been shown to contain cellulose (Gooday, 1971; Lewin, 1958), so it seems probable that the ability to use cellulose evolved several times in algal evolution, as it has in other protistan groups (i.e. *Acetobacter*, cyanobacteria, fungi, chrysophytes...). Hydrolysis with 67% H_2SO_4 yielded only glucose remnants, suggesting that the cellulose of these four algae was relatively pure, and no other contaminating polymers like chitin, lignin or sporopollenin were found.

The amount of pentose (xylose and arabinose) in the walls separated the test organisms into two groups. *K. flaccidum* possessed almost 5 to 10 times the amount of pentose in any other test alga. This may be due to *K. flaccidum*'s high hemicellulose content or the abundance of pentose in the water soluble sheath. The absence of such high levels of pentose from *U. belkae* suggests that the water soluble sheaths of *U. belkae* and *K. flaccidum* may be quite different.

3. Other Components

From acetolysis analysis of the test organisms, sporopollenin was found to be absent. Sporopollenin, a long chain secondary carotenoid (Atkinson et al., 1972), is found in various groups of differing evolutionary lines in algae (e.g. Chlorococcales), as well as in land plant pollen grains. It may be that this substance is a cell wall constituent in those terrestrial organisms which have become persistent (instead of transient) types or have propagule dissemination by means other than water. Lignin was not found in any test alga.

4. Distinction of Three Wall Types: Relation to Recent Phylogenetic Proposals

The theory of three possible lines of evolution in the green algae (Mattox and Stewart, 1977; Stewart and Mattox, 1978) suggested by ultrastructural data is supported by the present biochemical data from cell wall studies. In the Ulvaceae, our work and that of others (Percival and McDowell, 1967) shows that there exists a high amount of cellulose (as compared to other green algae, except for members of siphonaceous green algae), high levels of water soluble rhamnose, low percentages of water soluble uronic acid, low pentose and a wall protein that contains little hydroxyproline, and a questionable glycan region. By its slow electrophoretic movement, this protein is perhaps larger in molecular weight than the wall proteins of other algae studied. *P. terrestre* and *U. belkae* are chlorophycean green algae and resemble each other in wall analyses. They both possess low pentose, low water soluble rhamnose, high water soluble uronic acid content, unlike other tested chlorophycean green algae (Kuo-shii and Barber, 1975), and cellulose between 12 and 14%. They have a true hydroxyproline glycoprotein in the wall which separates into five bands of medium mobility (as compared to other test algae). *K. flaccidum*, a charophycean green alga, has a hydroxyproline glycoprotein similar to that found in land plants (Albersheim, 1976). Its mobility upon electrophoresis is fast and hence its subunits or glycoprotein fragments may be of lower molecular weight than the proteins of algal walls of other evolutionary lines. *K. flaccidum* has low amounts of cellulose, high hemicellulose and pentose content,

suggesting that it may not be on the direct line to higher plants, rather it may be a side branch member. Its low uronic acid content and level of water soluble rhamnose is comparable to that found in land plants.

5. *The Evolution of the Cell Wall in Green Algae*

Cell wall proteins, their structure and function, have only recently been investigated in green algae, as has the polysaccharide content of freshwater and terrestrial algae that may be early precursors to land plants and higher green algae. Results from the study of cell walls need to be compared with the chemical characteristics of scales of primitive green flagellates, but little is known about the extracellular constituents of prasinophytes and other primitive green algae. Reports of chemical analyses performed on the thecae of *Platymonas* and walls of *Chlamydomonas* (Catt et al., 1976; Kuo-shii and Barber, 1975; Roberts, 1974; Horne et al., 1971; Miller et al., 1972; Lamporte and Miller, 1971; Lewin, 1958; Gooday, 1971) show that these extracellular coverings are made up of polysaccharides and glycoproteins. Preliminary evidence from our current investigations shows that proteins are found in prasinophyte scales. Manton and Parke (1965) present evidence that thecae are fusion products of scales in *Platymonas* and *Prasinocladus*, and the work of optical transforms by Roberts (1974) on

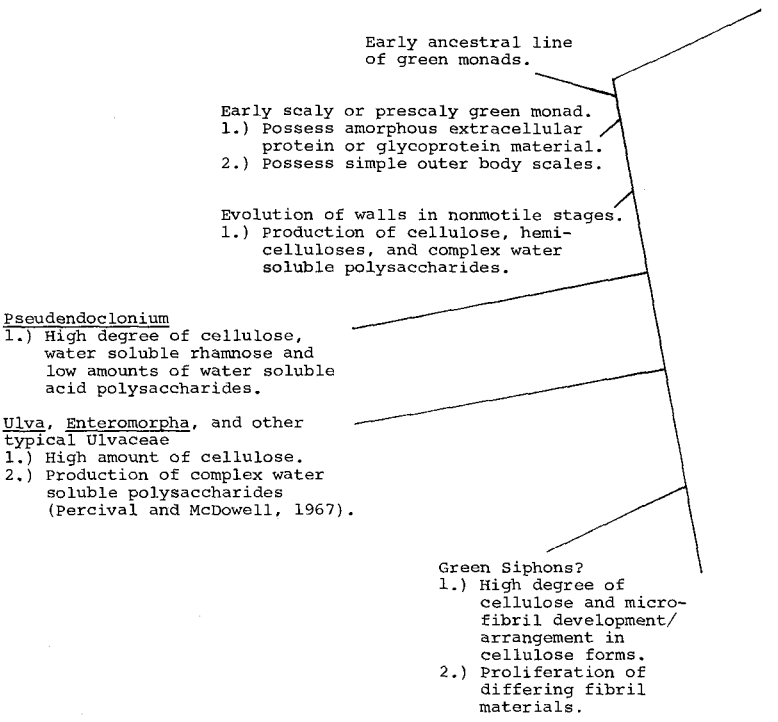

Fig. 1. Theoretical phylogenetic scheme for the evolution of the Ulvaceae, based upon preliminary wall studies and ultrastructure of cytokineses and mitoses (Mattox and Stewart, 1977; Stewart and Mattox, 1978). [The genera in these schemes (Fig. 1, 2, 3) are suggested extant forms, and are not necessarily the actual organisms where evolution diverged. The positions indicated by the extant organisms are suggested and need not be quantitative placements.]

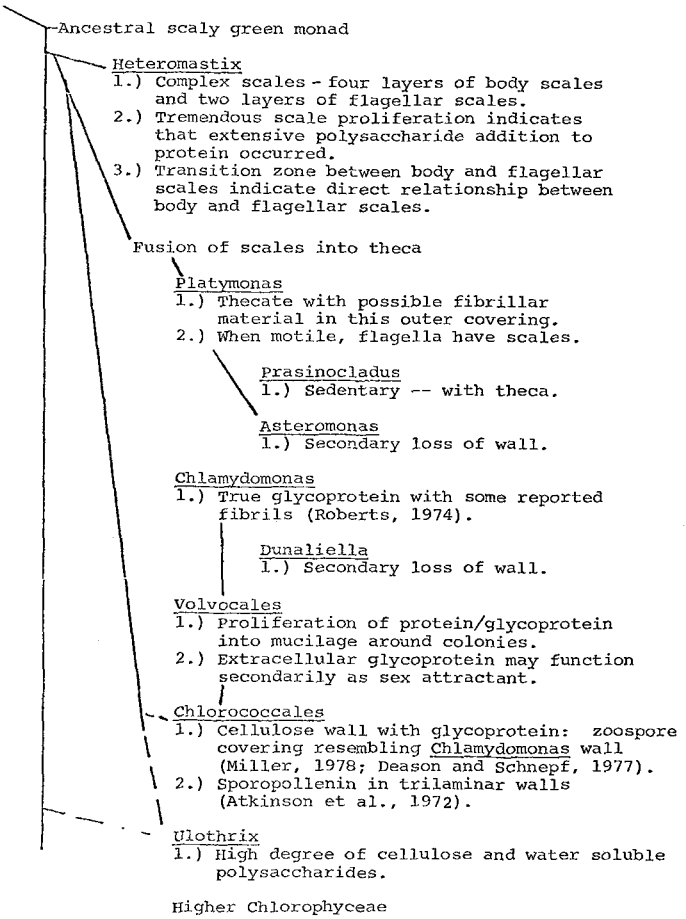

Fig. 2. Theoretical phylogenetic scheme for the evolution of the Chlorophyceae, based upon preliminary wall studies and ultrastructure of cytokineses and mitoses (Mattox and Stewart, 1977; Stewart and Mattox, 1978)

more advanced algal 'thecae' or walls (i.e. *Chlamydomonas* species and other volvocalean algae) along with our electron diffraction studies (unpublished) show that scales and thecae are chemically similar. Complete chemical analyses must be made on scales, and we are actively pursuing that aim. Such data must be available before any definite conclusions can be made, but it is of interest to discuss the origin of the cell wall in green algae (see Figs. 1, 2, 3), based upon evidence of recent research, both ultrastructural and biochemical.

It is very probable that early in the prasinophyte line, cells lacked defined outer coverings (i.e. scales or walls), but possessed a thin 'skin' of mucilage, which very likely was proteinaceous or glycoproteinaceous in nature. Many naked protistan cells or mucilage-covered cells with ancestral nakedness secrete proteins or polysaccharides associated with protein around their plasmalemma (i.e. *Porphyridium*, yeasts,

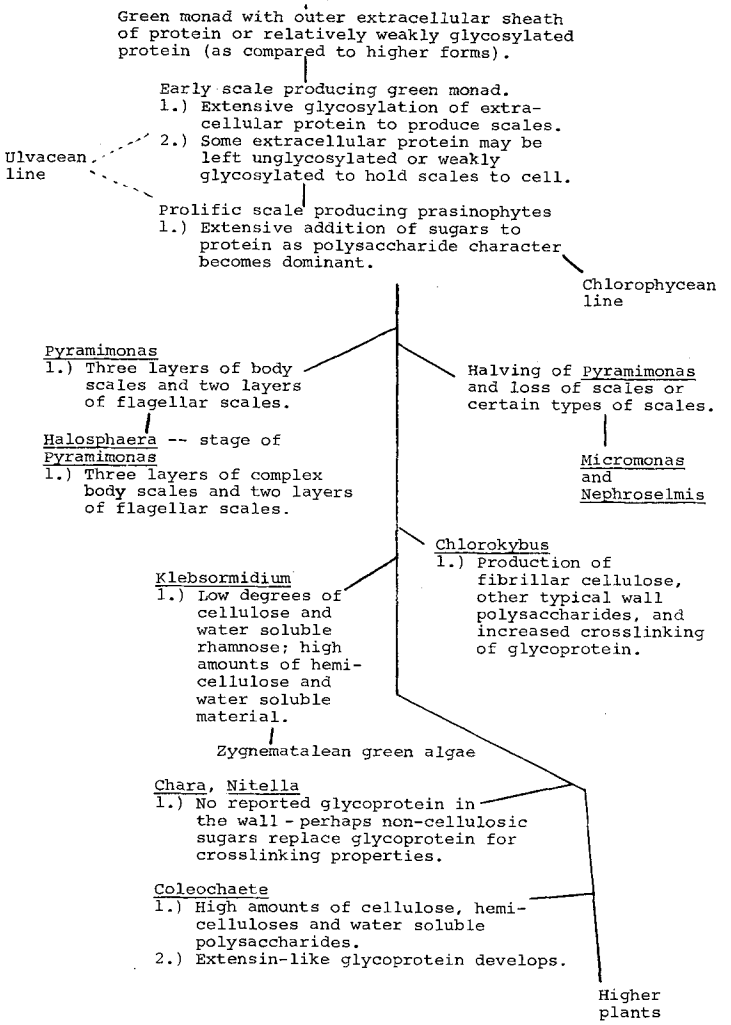

Fig. 3. Theoretical phylogenetic scheme for the evolution of the Charophyceae, based upon preliminary wall studies and ultrastructure of cytokineses and mitoses (Mattox and Stewart, 1977; Stewart and Mattox, 1978)

Bangiophycean red algae, protozoans). Transmission electron microscopic staining of *Pedinomonas* cells (Manton and Parke, 1960) indicates that the outer thin mucilage 'skin' found around the cell is probably proteinaceous.

During the evolution of early photosynthetic prasinophytes, as sugar probably became more abundant and/or varied, more saccharides may have been incorporated into the outer wall material, as a sort of structural reinforcement. Glycosylation of proteins (including enzymes) has been considered to be a protective or structural

device, especially in relation to wall proteins (Matile, 1975). With initial proteinaceous outer material serving as a structural template, increased amounts of sugars may have been added to the extracellular coverings, hence forming the glycoprotein and rigid scale-like material exterior to the cell. At this point, early in the origin of prasinophytes, scaly organisms may have branched off to later give rise to the ulvacean algae (Fig. 1). The lack of a true glycoprotein in *Pseudendoclonium* and relatively small amounts of protein in *Ulva* (Percival and McDowell, 1967) suggests a primitive wall condition in respect to the protein and extensive glycosylation. In this line, typical walls of advanced members developed during nonmotile phases. Cellulose, hemicelluloses and complex water soluble polysaccharides developed in this line, which includes *Pseduendoclonium, Ulva, Enteromorpha* and *Monostroma*.

In other lines of scaly green monads, extensive addition of various sugars to extracellular coatings (and perhaps inorganics) may have caused numerous and variant scales to develop (*Heteromastix, Pyramimonas, Mesostigma*). The inherent nature of the increasingly sugar-polymerized material or the qualitative alteration of the material may have become dominant in this scale structure. In one line of green algal evolution, specifically the chlorophycean line, a scaly green monad such as *Heteromastix*, may have undergone significant glycosylation and polymerization so that further polymerization of the polysaccharides may have caused fusion of the scales into a theca, or a change in structure may have aggregated the scales into a coherent structure followed by reinforcing polymerization. Finally, the carbohydrate material increased in quantity and form and typical chlorophycean green algae evolved (*Pleurastrum, Ulothrix*) (Fig. 2).

On another line of evolution, an organism like *Pyramimonas*, in a nonmotile phase such as in a *Halosphaera*-stage, may have developed a wall either from the interscaly amorphous material or from similar material between the scales and plasmalemma. From this point, typical charophycean and land plant walls developed (Fig. 3). Hence the glycoprotein would now serve as the crosslinking extensin found in higher plants (Albersheim, 1976; Lamporte and Miller, 1971). Many exceptions however show that variations have occurred. In *Chara* and *Nitella*, for example, no hydroxyproline-containing glycoprotein is reported from the wall. Perhaps this indicates a secondary loss of this protein and the replacement of the crosslinking function to noncellulosic polysaccharides.

The incorporation of cell wall analyses into phylogenetic studies of green algae and land plants has only just begun. When detailed ultrastructural, genetic and biochemical information about extracellular coverings becomes available, then more precise placing of taxons into phylogenies will come about.

References

Albersheim, P. (1976). The Primary Cell Wall. In: Plant biochemistry, J. Bonner, ed., pp. 226–274. New York: Academic Press

Atkinson, A.W., Gunning, B.E.S., John, P.C.L. (1972). Planta **107**, 1–32

Catt, J.W., Hills, G.J., Roberts, K. (1976). Planta **131**, 165–171

Deason, T.R., Schnepf, E. (1977). J. Phycol. **13**, 218–224

Gooday, G.W. (1971). J. Exptl. Bot. **22**, 959–971

Gotelli, I., Cleland, R. (1968). Am. J. Bot. **55(8)**, 907–914
Herth, W., Kuppel, A., Schnepf, E. (1977). J. Cell Biol. **73**, 311–321
Horne, R.W., Davies, D.R., Norton, K., Gurney-Smith, M. (1971). Nature **232**, 1193–1195
Jensen, W.A. (1962). Botanical histochemistry. pp. 1–408. San Francisco: W.H. Freeman
Keleti, G., Lederer, W.H. (1974). Handbook of micromethods for biological sciences. pp. 36–49. New York: Van Nostrand, Reinhold
Keyser, J.W. (1964). Anal. Biochem. **9**, 249
Kuo-shii, J., Barber, G.A. (1975). Phytochem. **14**, 2459–2461
Lamporte, D.T.A., Miller, D.H. (1974). Plant Physiol. **48**, 454–455
Leach, A.A. (1960). Biochem. J. **74**, 70–71
Lewin, R.A. (1958). J. Gen. Microbiol. **19**, 87–90
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. (1951). J. Biol. Chem. **193**, 265–275
Manton, I., Parke, M.J. (1960). J. Mar. Biol. Ass, U. K. **39**, 275–298
Manton, I., Parke, M.J. (1965). J. Mar. Biol. Ass. U. K. **45**, 743–754
Matile, Ph. (1975). The lytic compartment of plant cells. pp. 1–10. Berlin-Heidelberg-New York: Springer
Mattox, K.R., Stewart, K.D. (1973). Can. J. Bot. **51(7)**, 1425–1430
Mattox, K.R., Stewart, K.D. (1977). Am. J. Bot. **64**, 931–45
Miller, D.H. (1978). J. Phycol. **14**, 189–194
Miller, D.H., Lamporte, D.T.A., Miller, H. (1972). Science **176**, 918–920
Northcote, D.H., Goulding, K.J., Horne, R.W. (1958). Biochem. J. **70**, 391–397
Percival, E., McDowell, R.H. (1967). Chemistry and enzymology of marine algal polysaccharides. pp. 1–219. London and New York: Academic Press
Preston, R.O. (1975). The physical biology of plant cell walls. pp. 32–276. London: Chapman and Hall
Roberts, K. (1974). Phil. Trans. Roy. Soc. Lond. Ser. B **268**, 129–146
Spiro, R.G. (1966). Methods in enzymology. Vol. 8. Colowyck, S. and Kaplan, O., eds., pp. 1–50. New York: Academic Press
Stewart, K.D., Mattox, K.R. (1975). Bot. Rev. **41**, 104–135
Stewart, K.D., Mattox, K.R. (1978). Biosystems **10**, 145–152
Swain, T. (1972). Biochemical evolution in plants. In: Molecular evolution II, biochemical evolution and the origin of life, E. Schoffeniels, ed., pp. 283–289. North Holland: North Holland
Undergraff, D.M. (1969). Anal. Biochem. **32**, 420–424

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