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Light and Electron Microscope Study of Cell Walls of Brown and Red Algae

Abstract. A survey of the structure of the cell walls of green, brown, and red algae, as seen under light and electron microscopes is in progress. In this report a comparison of the cell wall structure of a brown alga, *Dictyota flabellata*, and a red alga, *Helminthocladia californica*, is presented. In *Dictyota*, typical of the brown algae, the microfibrillar pattern in the apical cells and in the adjacent cells of the thallus tip is reticulate. In mature cells the microfibrils are dominantly parallel in orientation. Pits, fields of closely set pores, are distinctive. The microfibrils in the pit areas are masked by nonfibrillar material. *Helminthocladia*, with a cell wall characteristic of the red algae, differs from *Dictyota* in that the microfibrillar pattern is reticulate throughout the thallus. In the pit areas the microfibrils are not masked by amorphous material.

Introductory electron microscope studies began with an examination of the cell wall of the green alga, *Valonia* (*1*). Later work on other species of the Chlorophyta demonstrated a great variability in the structure of the wall (*2*). On the basis of the crystalinity of cellulose, three classes were recognized in the group as a whole (*3*). Detailed electron microscope reports on cell wall structure in the brown and in the red algae are comparatively few; among them are the papers of Cronshaw *et al.* (*4*) and Myers *et al.* (*5*).

The specimens of brown and red algae were collected on the coast of southern California in tide pools of the littoral zone and also during skin-diving expeditions in the sublittoral zone to depths of 40 feet. In the present report the structure of the walls of the brown alga *Dictyota flabellata* and the red alga *Helminthocladia californica* is compared. The two species examined appear to be characteristic of their respective groups. So far as we are aware, they have not been previously described.

Dictyota flabellata, a member of the order Dictyotales (*6*), is a smooth-margined, dichotomously branched brown alga which grows attached to rocks in tide pools and to depths of about 40 feet (*7*). It is a low-growing Phaeophyte with blades up to 15 cm long, 3 cm wide, and approximately 150 μ thick. As is characteristic for the order, the blade possesses apical growth. In *Dictyota* a single lens-shaped apical cell with a thick outer wall cuts off one cell which then undergoes enlargement and anticlinal septation, forming rows of cells radiating from the apex (*6*). The mature blade, as seen in transverse section, is three cell layers thick. The upper and lower layers consist of cuboidal cells about 20 μ deep. The central layer consists of larger rectangular cells, about 100 μ long, 45 μ wide, and 80 μ deep. Intercellular spaces occur at the cell corners. In the large central cells, pit fields are visible under the light microscope on all cell faces where cell walls are in contact. Preliminary microchemical tests indicate that the cell wall consists of cellulose (I_2KI and H_2SO_4, 80 percent) and pectic materials (Ruthenium red).

For study under the electron microscope the first millimeter of the young blade tip, which includes the apical cell, was isolated by dissection and then cleared of noncellulose material by treatment in a 1:1 solution of 10 percent nitric acid and 10 percent chromic acid at a temperature of 20°C for 2 to 3 hours. After 6 to 10 washings in distilled water, the fragments were ultrasonically macerated at 1 Mcy/sec for 30 seconds. Drops of this suspension which yielded whole cells, cell fragments, and clumps of cells were then placed on Formvar-coated grids and shadowed with palladium.

In the walls of the apical cell and the adjacent cells of the growing tip, the microfibrillar pattern is reticulate. Pores, groups of pores, or pits (*8*) are evident in the loose microfibrillar network. In fragments where clearing is not complete, plasmodesmata are evident in pores and pits. Thickening of the cell wall is evident in the increasingly larger cells in the first 300 μ of the thallus tip. The microfibrils, ranging in diameter from 100 to 250 angstroms, are deposited in parallel orientation and effectively mask the primary reticulate wall pattern except in specialized pit field areas. In these areas the microfibrils are masked by nonfibrillar material (Fig. 1). In torn fragments of pit areas, however, the underlying microfibrils are visible.

Helminthocladia californica, a red alga, a member of the order Nemalionales (*6*), also occurs attached to rocks in upper intertidal pools (*7*). The mucilaginous thallus is irregularly and indeterminately branched and may reach a length of 15 cm. Under the light microscope the thallus is seen to be of multiaxial construction with a medulla of interwoven, septate, branched filaments, ranging from 5 to 25 μ in diameter, which terminate in an outer coating of filament tips forming the cortex. The filaments increase in

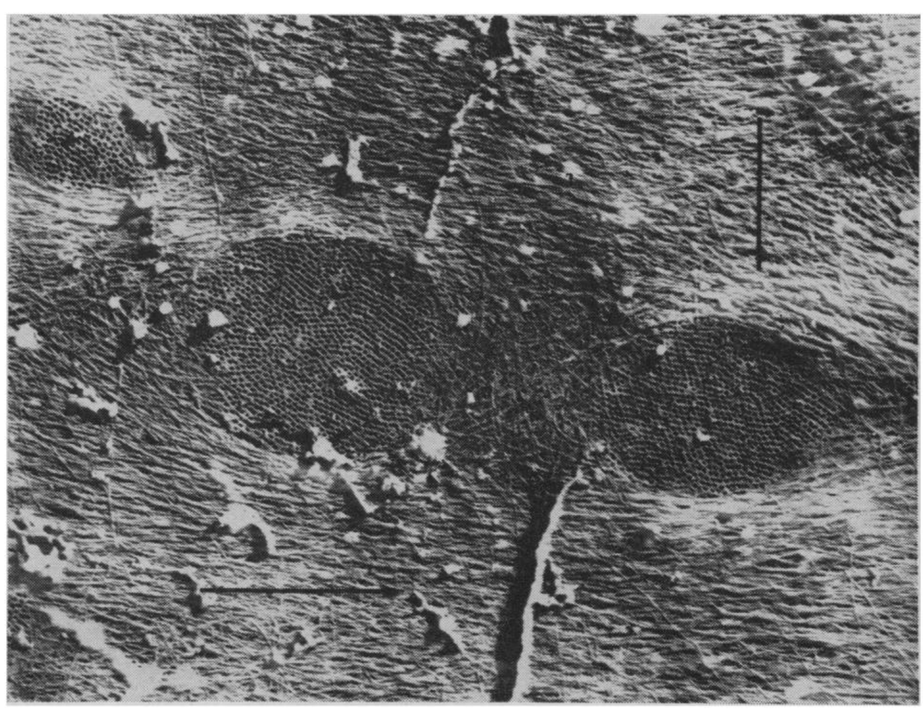

Fig. 1. Portion of the wall of a large central cell of *Dictyota flabellata*. Two large pits are flanked by microfibrils with a dominantly parallel orientation and are separated by an area in which the fibrils retain the reticulate pattern. The arrow indicates the axis of the cell wall. Scale, 2μ.

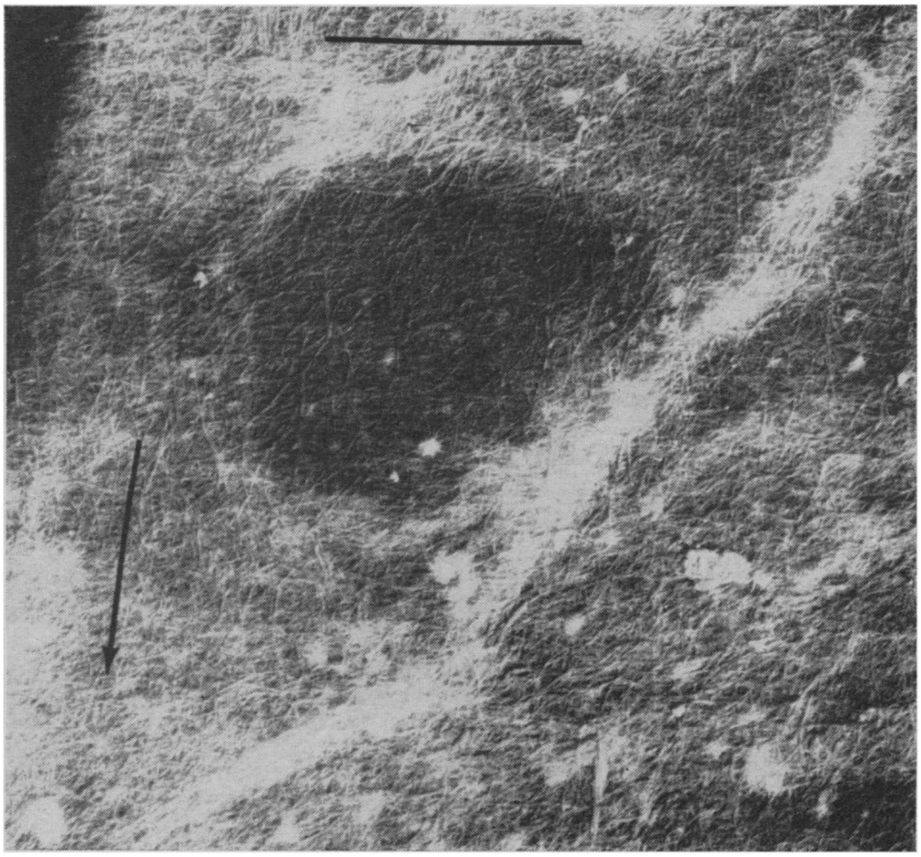

Fig. 2. Portion of a filament of *Helminthocladia californica*. The microfibrillar pattern of the cell wall and the pit base, as a whole, are reticulate. The arrow indicates the axis of the filament. Scale, 2μ.

length by apical growth. Protoplasmic connections are visible where the septa of the filaments are in contact. Other than these primary pit connections, no pitting is visible under the light microscope (*6*). Preliminary microchemical tests indicate the presence of pectic substances throughout the entire thallus. The reaction for cellulose is positive, and cellulose occurs both in the cell wall and in the mucilaginous sheath which surrounds the filaments.

Under the electron microscope, the reticulate microfibrillar pattern of the cell wall is clearly evident both in chemically cleared and in fresh material (Fig. 2). Although the filaments increase in length by apical cell growth, no difference in microfibrillar pattern or pitting has been observed between the older filaments of the medulla and the younger cells of the cortex. In addition to the pit connections of the perforate septa, termed primary pits by Fritsch (*6*), a second type of pitting is found on the radial walls where two filaments are in contact (Fig. 2). In these pit areas, actually thin areas in the loosely woven microfibrillar layer, the microfibrils are not masked by amorphous material.

In conclusion, the cell walls of brown and red algae examined consist of microfibrils with a diameter range of 100 to 250 angstroms. So far as the present survey indicates, a high degree of uniformity of microfibrillar orientation and pitting exists throughout the brown algae as represented by *Dictyota* and the red algae as represented by *Helminthocladia*. The two types differ as to the orientation of the microfibrils and the type and distribution of pitting (*9*).

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Effect of Synthetic Polylysine on Fungi

Abstract. The synthetic, basic poly-α-amino acid, polylysine, had antifungal activity against plant pathogens (three strains of fusaria, three isolates of verticillia, and *Ceratocystis fimbriata*) and against the human pathogens (*Trichophyton mentagrophytes, T. rubrum*, and *Candida albicans*) in vitro. It inhibited penetration of *Ceratocystis fimbriata* on sweet potato slices. Polylysine inhibited the infection of tomato cuttings by *Fusarium oxysporum* f. *lycopersici*, but it was also toxic to the plants.

Several types of infective agents are inhibited by the synthetic basic poly-α-amino acid of lysine. Polylysine reduced the infectivity of tobacco mosaic virus; protected chick embryos against infection with animal viruses such as mumps, infectious bronchitis, Newcastle disease virus, and influenza B virus; and inhibited multiplication of bacteriophage (*1*). More recently polylysine has been shown to exert an antibacterial effect against certain bacteria both in vitro and in vivo (*2*), and to increase survival in mice bearing certain ascites tumors (*3*). This paper reports the effect of polylysine on the growth and invasiveness of certain fungi pathogenic to plants or human beings.

Polylysine was prepared by an ammonia-initiated polymerization of ϵ-carbobenzoxy-L-lysine N-carboxy anhydride in dioxane, in which the molar ratio of anhydride to ammonia was 20:1 (*4*). The polylysine was added to Czapek's salt solution (*5*), which contained 51 g of glucose per liter, to give a final concentration of polylysine ranging from 1 to 100 μg/ml. The flasks were inoculated with a heavy inoculum of spores; those inoculated with fusaria were incubated at 28°C; those with verticillia, at 21°C. A semisynthetic medium (*6*) was used for the experiments with *Ceratocystis fimbriata* Ell. and Halst. and was incubated at 28°C.

Polylysine (100 μg/ml of medium) inhibited the growth of *Fusarium oxysporum* f. *conglutinans* (Wr.) Synd. and Hans., *F. oxysporum* f. *cubense* (E.F.S.) Synd. and Hans., and *F. oxysporum* f. *lycopersici* (Sacc.) Synd. and Hans. for 2½ weeks; of *Verticillium albo-atrum* Reinke and Berth. isolate 4 (T-16) (*7*) for 3 weeks; and of *V. albo-atrum* isolate 50 for 2 months.

Verticillium albo-atrum isolate 1 and