

Visualization of cell wall architecture of *Oocystis apiculata* by using of rapid-freezing and deep etching techniques*¹

Takeshi FUJINO*² and Takao ITOH*²

(Received June 1, 1994)

Keywords : cell wall, cellulose microfibril, cross-bridge, *Oocystis apiculata*.

In this study, molecular architecture of the cell wall was investigated using rapid freezing and deep etching techniques. These techniques allow us to observe the three-dimensional ultrastructure of the cell wall faithfully at high resolution.

A fresh water green alga, *Oocystis apiculata*, was used because it has large cellulose microfibrils compared to land plants. This feature makes it easier to distinguish from the other cell wall components. Using the cell wall of *O. apiculata*, we observed alteration of the cell wall morphology after selective chemical extraction, in order to elucidate the three-dimensional architecture of the cell wall components.

Firstly, we observed the intact cell wall of *O. apiculata* by deep etching technique. The results obtained are as follows; (1) the inner wall was composed of anastomosing structure of non-cellulosic components (il in Fig. 1); (2) the middle layer was polylamellated and composed of criss-crossed cellulose microfibrils with the mean diameter of 16.8 nm (ml in Fig. 1), there was innumerable and very thin strands that cross-bridged among the cellulose microfibrils especially in the middle layer of the inter-cellulose microfibril space (arrowheads in Fig. 1); (3) the outer layer had fibril-like protuberances, where the diameter was approximately 6.4 nm. The intercellular region was occupied with the thin fibrils in random network and the mean diameter of them was 6.4 nm.

Secondly, we applied EDTA, 4% KOH and 24% KOH for graded extraction of treatment. In EDTA treatment, no morphological change was observed. However, in 4% KOH treatment, fibrils which showed random network in intercellular region were completely disappeared and only the surface of the outer layer showed fibrillar structure. Subsequently in 24% KOH treatment, cellulose microfibrils were found to be assembled into thick band. However thin strand that cross-bridged among cellulose microfibrils were

*¹ A part of this work was presented at the 44th Annual Meeting of the Japan Wood Research Society in Nara, 1994, April.

*² Laboratory of Cell Structure and Function.

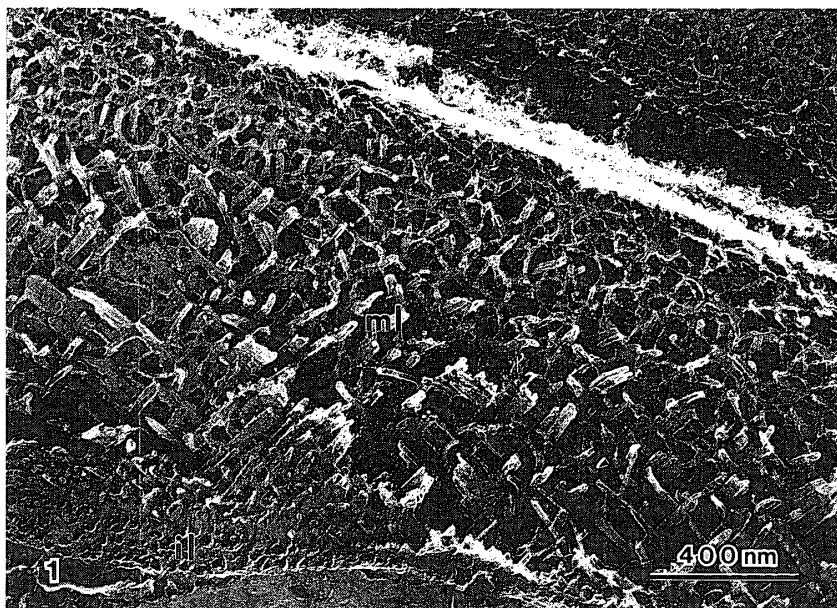


Fig. 1. Deep-etched image of the cell wall of *O. apiculata*. The wall consisting of inner and middle layers is shown. Cross-bridges between cellulose microfibrils are observed in the middle layer (arrowheads). il, inner layer; ml, middle layer.

still remained.

Finally, extract treatment was carried out using an acetic/nitric acid reagent (AN). All the components except cellulose microfibrils were disappeared, so that the cellulose microfibrils in the middle layer were aggregated into a stuck form.

From all the results mentioned earlier, it is suggested that the network of the filamentous substance that occupied the intercellular region is 4% KOH soluble polysaccharides. Particularly, from the effects of each treatments, it is presumed that the thin strands which cross-bridged with cellulose microfibrils in the middle layer is non-cellulosic substance. This is because the thin strands are extremely different in diameter from cellulose microfibrils in the middle layer and they disappeared in AN treatment. Furthermore, thin strands were observed to be existing after 24% KOH treatment with cross-bridging among cellulose microfibrils even though cellulose microfibrils were found to be assembled during swelling with strong alkali treatment. Consequently after 24% KOH treatment, the middle layer still maintained their own three-dimensional architecture by keeping the inter-cellulose microfibril space.

Therefore, The cross-bridging substance may be hemicellulosic polysaccharide and resistant to alkali in nature. It can be suggested that these hemicellulosic substances would be involved in keeping three-dimensional architecture of the cell wall because these substance can be removed only by AN treatment and then cellulose microfibrils were assembled into a stuck form.