pH-Dependent Maintenance of Cell Wall Integrity in the Giant-Celled Green Alga Valonia utricularis

Ichiro Mine¹*, Sho Suzuki², Kun-Feng Li¹ and Satoko Sekida¹

¹Graduate School of Kuroshio Science, Kochi University, 2–5–1 Akebono-cho, Kochi 780–8520, Japan
²Department of Biological Science, Faculty of Science, Kochi University, 2–5–1 Akebono-cho, Kochi 780–8520, Japan

Received August 13, 2017; accepted October 27, 2017

Summary It has been empirically known that cell walls isolated from giant-celled marine algae, such as Valonia spp., become liable to separate into several layers when transferred from seawater to a diluted buffer, indicating that the cell wall integrity is affected by environmental factors. We established an experimental system for a quantitative evaluation of the separation and investigated the effect of pH on the maintenance of cell wall integrity in V. utricularis. Cell wall strips incubated in artificial seawater (ASW) containing inorganic salts did not separate, whereas all strips incubated in distilled water separated under ultrasonication. Observations by transmission electron microscopy showed that there were gaps between cell wall layers in the cell walls incubated in distilled water; such gaps were not observed under ASW incubation. Separation experiments on cell wall strips incubated in 20 mM Tris or MOPS solutions at various pH showed that the separation of cell wall layers was dependent on environmental pH; namely, they were likely to separate in acidic conditions but not in alkaline conditions. The pH of 20 mM Tris and MOPS at which 50% of all cell wall strips separated into layers was estimated to be 8.4 and 9.2, respectively. These results suggest that the cell wall integrity of the alga is maintained by ionic bonds between the cell wall layers, and the major inorganic ions in seawater might contribute to binding of the cell wall layers, thus preventing separation.

Key words Cell wall matrix component, Cellulose microfibril, Ionic bond, Ultrasonic treatment.

Cells of plants, including seaweeds, are surrounded by cell walls that have a certain mechanical strength. The swelling of the protoplasm through turgor pressure is suppressed by the tension generated along the surface of the cell wall, which is required for maintenance of tissue shape and extension or growth of the cells in the plant (Niklas 1992). The cell walls also play a role in protecting the plant body by mechanical and/or physiological defenses against the invasion of external microbes (Lagaert et al. 2009). From the viewpoint of technical problems in plant cell biology, cell walls have been considered an obstacle to introduction of macromolecules, such as antibodies, into the cell; therefore, the cell walls were removed enzymatically during specimen preparation in some methods of cell biology (Goodbody and Lloyd 1994).

In the giant-celled algae, on the other hand, cell walls have been removed by manual dissection under a dissecting microscope (Okuda et al. 2016). It has been empirically known by laboratory workers that the cell wall fragments removed from the cell of giant-celled green algae such as Dictyosphaeria cavernosa (Okuda et al. 1997a) and Valonia utricularis (Okuda et al. 1997b) separate into several layers if kept in diluted buffer solutions (unpublished observations). This suggests the presence of certain factors in seawater which are necessary for maintaining the cell wall integrity in these algae. To characterize these factors, an experimental system to quantitatively evaluate the cell wall separation in V. utricularis was established, and the effect of pH on the maintenance of cell wall integrity was investigated.

Materials and methods

The unialgal culture strain of V. utricularis (Roth) C. Agardh used in the previous study (Okuda et al. 1997b) was used in the present study. The algae were cultured in a half-strength PES medium (Provasoli 1966) and kept at 20±2°C and 12 h:12 h light/dark cycles with illumination ca. 4 W m⁻² in provided by cool fluorescent lamps. Artificial seawater (ASW) comprised 450 mM NaCl, 30 mM MgCl₂, 16 mM MgSO₄, 10 mM KCl, 10 mM CaCl₂, and 8.25 mM Tris–HCl (pH 7.8). Other experimental solutions were 20 mM Tris and 20 mM MOPS prepared to various pH levels from 5.5 to 11.0 by adding HCl or NaOH.

The cells were cut with a razor blade under a dissecting microscope and cell walls were isolated by removing the protoplasm using a hair of a painting brash. The isolated cell walls were cut into five strips, each 0.5 mm in width and 2–3 mm in length, and immersed in an ex-
Experimental solution at room temperature for 2 h. After incubation in the experimental solution, 1.0 mL of the solution containing the cell wall strips was transferred into a 1.5 mL Eppendorf tube and subjected to an ultrasonic treatment by a stainless-steel horn (3 mm in diameter) of an ultrasonic generator (VP-5, Taitec Corporation, Koshigaya). The tip of the horn was inserted 5 mm beneath the surface of the solution and the treatment was for 1 min under an output control (dial set at 25), to provide a constant force to separate the cell wall layers. After the ultrasonic treatment, the number of cell wall strips with separated layers was counted under a dissecting microscope.

After incubation in an experimental solution, some of the cell wall strips were fixed in a 0.1 M sodium cacodylate buffer (pH 7.2; CB) containing 3% glutaraldehyde for 1 h at room temperature, washed in CB, and post-fixed in CB containing 2% osmium tetroxide for 1 h at room temperature. The fixed samples were dehydrated in ethanol and embedded in LR White (London Resin). Thin sections were cut using a diamond knife on an ultramicrotome (UCT, Leica Microsystems, Wetzlar). The orientation of the embedded specimen in the specimen holder was adjusted under a dissecting microscope so that the plane of the cell wall was perpendicular to the direction of cutting. After transferring to a copper grid, the sections were stained with uranyl acetate and lead citrate, and observed with a JEOL JEM-1400 transmission electron microscope.

Results

Using the experimental procedure described above for the separation of cell wall layers, the effect of ASW in the experimental solution on the separation of cell wall strips into layers was investigated and the results are presented in Table 1. Incubation in distilled water caused cell wall strips to separate, whereas full-strength ASW did not cause cell wall separation. Although results were slightly more variable, approximately half of the strips treated in half-strength ASW were separated under sonication (Table 1).

Experiments conducted on the cell wall strips incubated in 20 mM Tris or MOPS buffers at various pH showed a strong dependence of the separation of cell wall layers on the environmental proton concentration as shown in Fig. 1. Cell wall separation was not observed after incubation in Tris buffer at pH 9.5 or higher, but all cell wall strips separated at pH 7.6 or lower. The pH at which 50% of cell wall strips are separated, as estimated from an approximation curve was ca. 8.4 (Fig. 1). However, the cell wall strips incubated in MOPS buffer separated at higher pH than those in Tris. The pH of 50% separation was ca. 9.2 and that at which no separation was observed was 10.7 or higher (Fig. 1).

Transmission electron micrographs of the cell walls incubated in full-strength ASW for 2 h, which did not show separation under experimental conditions, showed that the cell wall is composed of many layers flush...
against each other (Fig. 2a). Each cell wall layer contained cellulose microfibrils (CMFs), the direction of which was distinct between neighboring layers. The outer, earlier-deposited layers were thinner than the inner, newly deposited ones, likely due to the expansive growth of the cell. In contrast, gaps between neighboring cell wall layers were observed in the cell walls incubated in distilled water for 2 h, which was liable to separate into layers, in both the outer and inner regions of the cell wall (Fig. 2b).

Discussion

In the present study, we have shown that the separation of the cell wall isolated from V. utricularis into layers can be estimated quantitatively by our experimental system using ultrasonic treatment for applying constant force to the cell wall. Using this experimental system, it was clearly shown that the cell walls were liable to be separated under acidic rather than alkaline conditions. This implies that the cell wall integrity, or binding between cell wall layers, is dependent on the environmental pH. As shown in Fig. 1, the relationship between the frequency of cell wall separation and pH fitted to an approximation by a sigmoid curve. This might indicate that the cell wall layer has a threshold pH for binding with the neighboring layers, which was found to be 8.4 and 9.4 in 20 mM Tris and MOPS, respectively. The pH-dependent separation of the cell wall layers observed in the present study suggests that the binding between cell wall layers is maintained by some kind of ionic bonds between the surfaces of the neighboring layers. The pH of natural seawater in the ocean is stable at about 8 (Chester and Jickells 2012), and incubation in ASW did not cause cell wall separation although its pH was significantly lower than the buffer threshold pH as described above. Considering these facts, it is probable that the presence of certain inorganic ions included in ASW, such as Na\(^{+}\), K\(^{+}\), Mg\(^{2+}\), Ca\(^{2+}\), Cl\(^{-}\), and SO\(_4^{2-}\), would contribute to the maintenance of the cell wall integrity in addition to low H\(^{+}\) concentration through the strengthening of the ionic bonds between layers.

Observations of the cell wall fine structures showed that the cell wall of V. utricularis was composed of many layers distinctive in the directions of CMF arrangement. Although there were no significant changes in appearance each layer, gaps were formed between some cell wall layers which were liable to separate (Fig. 2b), indicating that the binding between cell wall layers was loosened by the separation treatment instead of destroying the constituents of each layer. Cell walls composed of many layers with distinct CMF directions have been reported in various taxa of land plants (Probine 1963, Vian et al. 1993) and macroscopic algae (Mine et al. 2015). The CMF arrangement in the cell wall layers is classified as a multinet arrangement in the characean algae and land plants, whereas it is a crossed-fibril type in Valonia and certain giant-celled green algae (Mine et al. 2015). In crossed-fibril cell walls, the CMFs in individual layers are oriented in a uniform direction and the relative angle of CMF between neighboring layers is approximately 100°. To the best of our knowledge, there have currently been no investigations on the binding between cell wall layers in both types of cell wall and the present study is the first report of the dependence of the binding on environmental conditions.

The cell wall structure(s) responsible for the binding between cell wall layers would possibly result from the interaction between various matrix components and/or CMFs. However, only a few reports on the matrix components in Valonia cell walls have been published. An earlier study on the fine structure of the cell wall of regenerating aplanospores in V. ventricosa observed by transmission electron microscopy on shadowed specimens indicated the presence of pectin-like amorphous materials that concealed the CMF network (Plate X in Steward and Muhlethaler 1953). Recently, thin fibers coiling around CMFs along the outer surface of the cell wall detected by atomic force microscopy were reported in V. ventricosa (Eslick et al. 2014) but it is not known if the fibers are present between cell wall layers. Observations of the fine structures of the surface of the separated cell wall layers would potentially be useful in understanding the structural components enabling binding between cell wall layers.

References


