Increase in Tension at the Surface of Protoplast as a Sign of Cell Wall Formation in *Boergesenia forbesii*

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Protoplasts prepared from thalli of *Boergesenia forbesii* were subjected to the measurement of tension at the surface by means of the suction method. The tension at the surface just after completion of spheration was 0.2-0.4 dyne/cm irrespective of the temperature. Since this value is of the same order of magnitude as those measured in other species of cells without a cell coat, it is suggested that the protoplast just after spheration is covered with the plasma membrane. The measured tension at the surface was constant and not affected by the degree of deformation of the protoplast, suggesting that the surface of the protoplast is not elastic.

After some time the tension began to increase abruptly. Both the latent time elapsed prior to the increase in the tension and the rate of tension increase were strongly dependent on the temperature. As long as protoplasts were treated with cellulase, increase in the tension was completely inhibited, but it occurred soon after washing out of the cellulase. Protoplasts were stained with Calcoflour White at around the time when the tension began to increase. These results suggest that the cell wall formation begins at the time of abrupt increase in the tension at the surface.

The vegetative thalli of *Boergesenia forbesii*, a giant alga which belongs to Siphonocladales, are well known, together with other coenocytic marine algae such as *Valonia*, *Bryopsis* and others, due to their large capacity to regenerate whole plants from many aplanospores which are produced inside the cell when the alga is subjected to mechanical stimulation. The formation of aplanospores inside the cell and their development to new plants or thalli in vitro have already been reported in detail by Enomoto and Hirose (1972). A similar observation was also made in the case of the protoplasts in vitro of *Bryopsis pulmosa* (Huds.) Agardh by Tatewaki and Nagata (1970). To induce formation of aplanospores, Enomoto and Hirose (1972) bathed the cell in twofold concentrated sea water or stimulated it by sticking it with a fine needle. Under these circumstances a thin layer of the protoplasm located at the inner surface of the cell wall begins to aggregate at different loci and finally produces many spherical protoplasmic masses or aplanospores whose diameters are 100-300 μm.

Similar aplanospore-like protoplasts can be obtained by culturing in vitro the protoplasmic masses which have been squeezed out into artificial sea water.

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It is supposed that the spheration is due to the change in ionic composition inside the cell. In a few hours after the formation of the proplasts the surface is covered with the cell wall. In a few days both aplanospores taken out from the mother cell and proplasts formed in vitro begin to germinate and grow up into normal cells when they are cultured in Provasoli’s ES-medium or in natural sea water (Enomoto and Hirose, 1972; Ishizawa et al., 1979). For the cell wall formation many processes are expected to occur not only in the cytoplasm but also in the newly formed cell membrane.

The process of spheration is believed to be an active process, since it is strictly dependent on temperature (Ishizawa et al., 1979). Changes in the mechanical properties of the surface membrane of Boergesenia proplasts may be detected by measuring tension at the surface (T), as in sea urchin eggs where T changes cyclically with the cell division cycle (Hiramoto, 1967; Yoneda and Dan, 1972) or in endoplasm drops of Nitella where development of T is closely related to appearance of the membrane excitability (Ueda et al., 1973). It is expected that T of the plant proplast begins to increase when the cell surface is covered with the cell wall, since the cell can develop turgor after cell wall formation owing to the increase in mechanical consistency. In the present work the authors measured T of proplasts and found that its increase was closely related to the cell wall formation.

Materials and Methods

Boergesenia forbesii (Harvey) Feldmann was supplied by Dr. Enomoto of the Marine Laboratory of Kobe University. The proliferation of the alga was performed by making aplanospores in mother cells and transferring them into the natural sea water or Provasoli’s ES-medium (ESP-medium; Provasoli, 1966) at 27 C. The light regime was 12 hr light and 12 hr dark. The light intensity was about 2,000 lux, from white fluorescent tubes. Cells about 2 cm long were used for preparation of proplasts. To obtain proplasts cells were cut with scissors into two pieces in the standard artificial sea water (standard ASW) which contained 490 mM NaCl, 10 mM KCl, 50 mM MgCl₂, 10 mM CaCl₂ and 5 mM Tris-HCl buffer (pH 8.0). The proplasm adhering to the inner surface of the wall of the cut half cell formed many proplasts.

The proplast, just after completion of spheration, was freed from the cell and subjected to the measurement of tension at the surface at the same temperature as before. As will be discussed later it is very probable that the proplasm, when it formed the spherical proplast, is covered with the regenerated plasma membrane. In Bryopsis the proplasmic fragments forms the plasma membrane completely within an hour at 25 C (Kobayashi and Saikawa, 1975; Kobayashi et al., 1976).

Tension at the surface (T) of the proplast was measured by the suction method using the cell elastimeter (Mitchison and Swan, 1954). The whole setup is diagrammatically shown in Fig. 1. It consisted of a glass micropipette and two micromanipulators. One manipulator (M₁) served to move the micropipette (m) and the other (M₂) served to produce a small suction pressure which sucked the proplast