Induction of a New Alkaline Band at a Target Position in Internodal Cells of Chara corallina

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Characean cells develop alternating alkaline and acid bands on their surface upon illumination. However, the mechanism of band formation is not fully understood. In the present study, we succeeded in inducing a new alkaline band at an original acid band in internodal cells of Chara corallina. Chloroplasts in an acid band were locally removed by wounding the cell in the absence of the cell turgor pressure. The chloroplast-removed area was observed as a white belt in a green cylindrical internodal cell. This internodal cell developed a new alkaline band on the surface at the chloroplast-removed area. The narrower the chloroplast-removed area, the less significant the extent of OH⁻ extrusion. This is the first success in inducing a new alkaline band at a target position in Characeae.

Keywords: Alkaline band — Chara — Chloroplast — Membrane potential — Photosynthesis — Wounding.

Abbreviations: APW, artificial pond water; BFS, band formation solution; E₁₉₅, membrane potential.

Introduction

The main inorganic carbon species is HCO₃⁻ in a neutral or slightly alkaline aquatic habitat. Since the substrate for carbon fixation in photosynthesis is CO₂, OH⁻ is inevitably generated by photosynthetic activity in cells of submerged aquatic plants living in such a habitat. Some higher plants, such as Elodea and Potamogeton that have polar leaves, extrude OH⁻ from the morphologically upper surface of the leaves (Prins et al. 1982). In characean cells, OH⁻ is extruded in limited areas called alkaline bands. At an area between alkaline bands, H⁺ is extruded, forming an acid band. Thus, alkaline and acid bands are alternatively formed on the surface of internodal and branchlet cells of Characeae (Spear et al. 1969, Lucas and Dainty 1977). However, we do not yet fully understand the mechanism of band formation in Characeae. We could thus create a new strategy for understanding the mechanism by which bands are formed. In the present study, we succeeded in inducing a new alkaline band at the original acid band by applying local wounding to internodal cells of C. corallina.

Results and Discussion

We reported the effect of aluminum on alkaline band formation in internodal cells of C. corallina (Takano and Shimmen 1999). During the study, we noticed that an alkaline band was formed at the area where chloroplasts had been detached. The chloroplast-detached area was observed as a small white belt. In Characeae, chloroplasts are attached to the cortical gel ectoplasm and do not change position. It was supposed that chloroplasts had been detached before or during isolation of cells from the culture. Alkaline bands were visualized by illuminating an internodal cell bathed in artificial pond water (APW) supplemented with 5 mg/100 ml phenol red and 0.5 mM NaHCO₃ (pH 6.5–7) (Fig. 1A). We examined 12 cells that had such chloroplast-detached area(s) for alkaline band formation. All cells formed an alkaline band at the chloroplast-detached area. The cell shown in Fig. 1A had four white belts lacking chloroplasts. Alkaline bands were formed at the all white belts (arrowheads).

We expected that an alkaline band may be induced at the acid band, if we remove the chloroplasts at the acid band. Chloroplasts at the acid band were locally removed as follows. After bands were formed under illumination, the position of alkaline bands of an internodal cell was recorded in a photograph. The internodal cell was placed on a polyacrylate bench and dried until the cell turgor pressure was lost. On the other hand, a strip of silk thread was prepared, and both ends of the thread were pulled by hand to apply strong tension. With the thread, the internodal cell was pressed at an acid band. By this procedure, any chloroplasts in the region were removed and a white chloroplast-free belt was formed. The cell was kept in APW at least overnight before use. The widths of the chloroplast-removed areas ranged between 0.7 and 1.5 mm. Although many cells died due to severe wounding, some cells survived. The surviving cells were subjected to the analysis of alkaline band formation.

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In Fig. 1B, an internodal cell was illuminated for about 20 min and the position of alkaline bands was recorded in a photograph (a). Arrowheads indicate where cells were wounded. After the internodal cell was kept in APW for 1 d, it was subjected to alkaline band formation. As expected, a new alkaline band formed at the chloroplast-removed area (Fig. 1B b). One original band near the left node became less significant after wounding. In other cells, some original alkaline bands disappeared after a new alkaline band was formed at the chloroplast-removed area by wounding. We examined 10 cells, and all cells formed an alkaline band where the chloroplast was removed.

In younger internodal cells, the position of alkaline bands sometimes migrates without any treatment; therefore, it is possible that the new alkaline band was formed where the chloroplasts were removed only by chance. It has been reported that the position of alkaline bands is stable in older cells that have precipitation of CaCO$_3$ at the alkaline bands (Lucas and Smith 1973). This tendency was also the case in our material (data not shown). We carried out experiments to make a chloroplast-free area at the acid band using older cells that had CaCO$_3$ crystals on the cell surface. Such cells always formed strong alkaline bands at the position of CaCO$_3$ precipitation upon illumination (Fig. 1C a). A white chloroplast-removed area was formed at the original acid region by wounding (arrowhead). When examined for alkaline band formation after a 1-d incubation in APW, a new alkaline band appeared at the very position (data not shown). After a 4-d incubation in APW, the cell again formed an alkaline band where the chloroplasts were removed (b). The original strong alkaline bands were maintained after wounding. Such experiments were repeated for 15 cells and an alkaline band was formed where the chloroplasts were removed.
in all cells. Occasionally, unstable small bands were observed even in old internodal cells with CaCO₃. In the cell shown in Fig. 1C, one strong band near the right node migrated during 4-d incubation in APW. A new alkaline band might have formed where the chloroplasts were removed, just by chance, even in old cells with CaCO₃ crystals. However, without exception, new alkaline bands were always formed at the chloroplast-removed areas, supporting our contention that chloroplast removal by wounding was the real cause for induction of new alkaline bands.

The capacity of the cell to form an alkaline band at the chloroplast-removed area was stable. When wounded cells were examined after 2 months, an alkaline band remained where the chloroplast had been removed (data not shown).

In the above experiments, the widths of chloroplast-removed areas were 0.7–1.5 mm. In the following experiment, internodal cells with chloroplast-removed areas of shorter widths were prepared. The widths of chloroplast-removed areas ranged between 100 and 500 μm (in 21 cells with CaCO₃ crystals at the alkaline bands). The narrower the chloroplast-removed area, the less significant the extent of OH⁻ extrusion (data not shown).

Occasionally, “white windows” were formed by wounding (Fig. 1D a, b). In this cell, two windows were formed. The one that opened at the lower side of the cell is not seen in this photograph. OH⁻ eflux occurred at both windows (b). These alkaline spots were unstable, and only appeared occasionally upon illumination (data not shown).

The effect of wounding on the membrane potential (Eₘ) was studied. Eₘ before wounding was −216±3 mV (n=7). Eₘ at the chloroplast-removed area was measured about 1 h after wounding. It was −32±3 mV (n=5). Since the width of the measuring pool was 3 mm and the widths of the chloroplast-removed areas were 0.7–1.5 mm, Eₘ measured should represent the average value of intact (chloroplast-lodged) and wounded (chloroplast-removed) areas. Eₘ measured 2 d after chloroplast-removal was 212±8 mV (n=5). Thus, electrogensis was severely disturbed just after wounding but recovered after incubation.

To our knowledge, this is the first success in inducing a new alkaline band at a target position of the original acid band. At the chloroplast-removed area, most chloroplasts had been dislodged, indicating that anchoring chloroplasts to the gel ectoplasm is not always necessary for extrusion of OH⁻ to form alkaline bands. It is suggested that OH⁻ generated at some other area that has chloroplasts is transported to the chloroplast-removed area by cytoplasmic streaming. Actin bundles responsible for cytoplasmic streaming are anchored at the inner surface of chloroplasts (Kamitsubo 1966, Nagai and Rebhun 1966, Kersey and Wessells 1976). When chloroplasts were detached, actin bundles were also removed. Actin bundles are regenerated at the inner surface of the cortical gel layer lacking chloroplasts after one to several days (Kamitsubo 1972). It was suggested that bundles of actin filaments were also regenerated in the present material, since directed movement of endoplasm was observed at the chloroplast-removed area.

OH⁻ ions were easily accessible to the plasma membrane at the chloroplast-removed area. The higher accessibility for OH⁻ to the plasma membrane might be a cause for induction of new alkaline bands. The flow of endoplasm might be affected more or less at the boundary of the chloroplast-removed area. This is also another reason why a new alkaline band is induced. Success in inducing a new alkaline band at a target position in C. corallina would offer a new strategy for analyzing the mechanism for alkaline band formation in Characeae.

Materials and Methods

Chara corallina was cultured as reported previously (Mimura and Shimmen 1994). Internodal cells were isolated and kept in APW containing 1 mM NaCl, 0.1 mM KCl and 0.1 mM CaCl₂ (pH about 5.6).

To examine the capacity of the cell to form alkaline bands, we used a band formation solution (BFS), which was prepared by adding 0.5 mM NaHCO₃ and 5 mg/100 ml phenol red to APW (pH 6.5–7) (Takano and Shimmen 1999). A polycrystalline block with channels was prepared. The channel was 3 mm wide, 3 mm deep and 80 mm long. An internodal cell was rinsed with BFS and then placed in the channel of the polycrystalline block. After the channel was filled with BFS, it was covered with a thin transparent polycrystalline. The polycrystalline block was placed in a transparent polycrystalline box, the bottom of which was covered with a thin layer of pure water. A lid was put on the box to prevent the solution in the channel from evaporating. The cell was illuminated with fluorescent lamps (200 μmol m⁻² s⁻¹ at the upper surface of the polycrystalline box). After illumination, a photograph was taken to record the position of alkaline and acid bands.

The membrane potential (Eₘ) was measured as reported previously (Shimmen 1997). Briefly, an internodal cell was partitioned into three pools and Eₘ at the central pool was measured by the K-anesthesia method. The width of the central pool was 3 mm. Average values of Eₘ are shown with SE.

References


(Received January 30, 2002; Accepted June 19, 2002)