Communication

Abscisic Acid Increases Terrestrial Plant Cell Resistance to Hydrostatic Pressure


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ABSTRACT

Cells of the terrestrial plant species bromegrass (Bromus inermis L.) are not naturally adapted to withstand the hydrostatic pressures encountered in aquatic environments. However, after treatment with the natural plant growth hormone abscisic acid (75 micromolar), bromegrass cells survived a hydrostatic pressure of 101.3 megapascals, approximating the limits of ocean depth (10,860 m). The increased resistance to hydrostatic pressure from 1 to 7 days of abscisic acid treatment paralleled the induced elevation of cell tolerance to freezing stress.

The distribution of organisms in terrestrial and aquatic environments is mainly determined by temperature, water availability, and pressure (4). Species are often exposed to more than one environmental stress and thus must develop mechanisms for resisting several stresses. The effects of ABA in eliciting adaptation of land plants to a variety of environmental stresses are well documented (1, 20). ABA significantly increases freezing, desiccation, and salt resistance in several plant species (5, 14, 20). Endogenous ABA may also play a regulatory role in cross-adaptation of land plants, in which preexposure to one stress confers resistance to another (14). Collectively, evidence suggests that ABA may function as a general stress hormone in terrestrial plants.

The site of freezing injury in plants is considered to be the plasmamembrane (11). High hydrostatic pressures also produce deleterious membrane responses similar to alterations observed in plant cells exposed to freezing. A 101.3 MPa pressure results in ordering of membrane bilayers to a level equivalent caused by a 15 to 25°C drop in temperature (7). The ordering of membrane bilayers by increasing pressure or decreasing temperature results in a phase transition of the lipid bilayer from a solid to a gel state in several systems (7, 8).

Evidence indicates a homeoviscous adaptation to hydrostatic pressure stress where the proportion of unsaturated fatty acids in membrane phospholipids increases to optimize membrane fluidity in deep sea bacteria (8). This adaptational strategy is analogous to the increases in unsaturation in membrane lipids observed to occur during low temperature-induced acclimation in several organisms, including plants, bacteria, and poikilothermic animals (8). Recent evidence indicates genetic regulation by hydrostatic pressure (3). However, the mechanism of hydrostatic pressure perception and signal transduction to elicit adaptive responses is unknown. In spite of the ecological significance of hydrostatic pressure to the vertical distribution of marine organisms, there are as yet no reports of hormonal induction of pressure tolerance in aquatic organisms.

Hardy plant cells increase in freezing tolerance over a period of several weeks when exposed to low (nonlethal) temperatures. Cells of hardy plants also increase in freezing resistance at room temperature (23°C) within several days upon exposure to low concentrations of ABA (5). Because freezing and hydrostatic pressure stress appear to produce a similar type of injury to membranes, this study explored the hypothesis that the mechanism of resistance to freezing stress may be related to hydrostatic pressure tolerance.

MATERIALS AND METHODS

Plant Material

A bromegrass (Bromus inermis Leyss cv Manchar) cell suspension culture (BG-970) was grown in modified Erickson's media containing 0.5 mg/L 2,4-D (16). A 1.75 mL packed cell volume inoculum size was used to achieve more linear growth throughout the treatment period. The suspension culture was maintained on an Eberbach reciprocating shaker at 110 rpm at 23°C in the dark and routinely transferred every 2 weeks. ±ABA (75 μM) purchased from Calbiochem was added to the medium and autoclaved at 121°C for 15 min. Previous studies (5) determined this ABA concentration to be optimal for induction of freezing resistance in bromegrass cell suspension cultures.

Bromegrass cells were treated with ABA during the linear growth phase and tested for freezing and pressure resistance at 1, 3, 5, and 7 d after treatment.

Stress Tests

Freezing Stress

Cells were harvested and washed with 200 mL double-distilled H2O (about 20 volumes) to remove possible cryoprotectants.
Table 1. Viability of Bromus inermis Cells after 12 h Exposure to Pressure with and without ABA for 7 d

<table>
<thead>
<tr>
<th>Pressure (MPa)</th>
<th>Viability Tests*</th>
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<tbody>
<tr>
<td></td>
<td>TTC 75 µM ABA</td>
</tr>
<tr>
<td>0.1</td>
<td>+ + +</td>
</tr>
<tr>
<td>25.3</td>
<td>+ + +</td>
</tr>
<tr>
<td>50.6</td>
<td>- + +</td>
</tr>
<tr>
<td>76.0</td>
<td>- + +</td>
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<tr>
<td>101.3</td>
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* + and − indicate viability greater or less than 50% of viability at 0.1 MPa.

The protective effects of the medium residue during freezing. Cell samples of 200 mg fresh weight were frozen in 10 × 75 mm glass culture tubes after removal of excess extracellular water by low-speed centrifugation as previously described (16). Samples were equilibrated in a Neslab LT-50 low temperature bath at −1°C, nucleated with ice crystals, and held overnight. The temperature was lowered the following day at a rate of 2°C/h to −10°C and 3°C/h thereafter. Cell sample tubes were removed each hour from −3°C to −33°C and thawed at 4°C for 24 h.

Hydrostatic Pressure Stress

To prepare cells and cell clumps for exposure to hydrostatic pressure, samples of ABA-treated and control cells were placed into a 1 mL disposable syringe with a 23 gauge needle. Excess air bubbles were removed and the needle was plugged with a size 00 rubber stopper. Syringes containing cell samples were placed into stainless steel pressure cylinders. Hydrostatic pressures were applied for various periods of time (3, 6, 9, and 12 h) with a barokam unit described by Morita (12). Compression and decompression to and from treatment pressures were attained within minutes. Pressure stress experiments were performed at 23°C.

Viability Tests

Viability was assessed with TTC2 and FDA.

TTC

Each 50 mg sample was incubated in a 1 mL solution of 0.5% TTC (modified from 24) for 25 h in the dark and sealed with a serum stopper. Excess TTC was then removed and the cells were extracted with 3 mL of 95% ethanol per vial. After complete extraction (about 3 d), 300 µL of the ethanol extractant were transferred to ELISA plates and read at 495 nm with a Biotek Microplate Autoreader EL 309. In each experiment, three samples were analyzed for each treatment and the experiment was repeated six times. Viability was expressed as the temperature at which 50% of the cells were killed, as determined by the TTC test.

FDA

A 5 mg/mL (in acetone) FDA stock solution was diluted to 0.01% (v/v) in culture media according to Widholm (19). Cells isolated with a 30 µm sieve were observed under UV-excitation using a 50 W vapor mercury lamp fitted with a DAP1 02 blue filter to estimate relative fluorescence intensity. Although a gradient of fluorescence was observed, only cells emitting the highest fluorescence intensity were considered viable. At least 100 cells were counted within each sample.

RESULTS AND DISCUSSION

The hydrostatic pressure tolerance of untreated bromegrass cells was established by measuring viability after exposure to 0.1, 25.3, 50.6, and 101.3 MPa pressure for 12 h (Table 1). Control cell survival significantly declined on exposure to 25.3 MPa pressure. In contrast, ABA treatment enhanced cell viability at all experimental pressures. The extent of ABA-induced pressure tolerance in this study compares with the upper limit (110.0 MPa pressure at ocean depths of 10,860 m) of hydrostatic pressure tolerance observed in marine plant species (12, 18).

At 101.3 MPa pressure, 3 h exposure reduced the viability of untreated control cells by more than 50%, whereas 100% of ABA-treated cells survived 9 hours of exposure (Fig. 1). After 12 h, the viability of treated cells declined to 27%, but was still greater than the 3% survival of control cells. To account for potential time-dependent anoxic effects, viability was expressed as a function of cell viability after exposure for the specified time periods in the barokam unit at 0.1 MPa,

![Figure 1. Viability over a 12-h period of 7-d-old bromegrass cells exposed to 101.3 MPa pressure with (ABA) and without (control) 75 µM ABA treatment. Viability is expressed as the percentage of cell viability at 0.1 MPa pressure at each sampling time. The values represent the transformed mean ± SD of three separate experiments.](https://www.plantphysiol.org)
23°C. Bromegrass cell resistance to both freezing and hydrostatic pressure increased with length of exposure to ABA (Fig. 2). Incubation of cells in ABA at 23°C induced a marked increase in freezing resistance from −5°C to −26°C within 7 d. Similarly, resistance to 12 h of 101.3 MPa pressure increased over the 7 d ABA treatment period.

Deleterious conformational changes in membrane proteins during freezing stress may cause loss of plasmamembrane function (2). Membrane protein/lipid interactions are disrupted by hydrostatic pressures in model membrane systems (13). Hydrostatic pressure also disrupts chemical equilibria, pH (10), and causes molecular volume reduction (12). These effects can cause numerous metabolic aberrations that have both functional and structural consequences (12, 18).

ABA, a hormone that normally occurs in plants, is also found in organisms such as fungi (20) and mammalian brain cells (6). However, its role, if any, in these organisms has not been elucidated. Marine organisms that migrate vertically are particularly subject to variation in both pressure and temperature. Under these conditions, constant adaptation to changing environmental conditions is required. A conservation of adaptive polymerization mechanisms to both low temperature and hydrostatic pressure was determined in 14 vertebrate species (15). A stress hormone such as ABA that rapidly regulates cellular responses could have survival value. It is not known whether ABA occurs endogenously or if it is involved in the stress adaptation of such organisms. Considering evidence of ABA regulation of both membrane lipid composition and state (9), it would be of particular interest to examine ABA levels in organisms exhibiting homeoviscous adaptation.

The mechanism(s) of ABA action in conferring hydrostatic pressure and freezing resistance were not addressed in this study, but the results demonstrate for the first time that a hormone can increase cell resistance to hydrostatic pressure stress. These results may have relevance in elucidating the mechanism of freezing injury, i.e., hydrostatic pressure could be used to simulate freezing injury responses without exposing cells to low temperature or ice formation. Finally, these results also raise the possibility that ABA may play a central regulatory role in the adaptation of terrestrial plants and marine organisms to a variety of environmental stresses.

ACKNOWLEDGMENTS

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LITERATURE CITED