Osmotic Shock Inhibits Auxin-stimulated Acidification and Growth

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ABSTRACT

Cells of oat coleoptiles (Avena sativa L. cv. "Garry") have been osmotically shocked in order to observe the effect of alterations of the plasma membrane on some auxin responses. When coleoptile sections were treated sequentially with 0.5 M mannitol and 1 mM Na-phosphate (pH 6.4) at 4 C, polar auxin transport and acidification by 1 mM CaCl2 were unaffected, but auxin-stimulated acidification and growth were eliminated. Shock treatment also had no effect on acid-stimulated growth or on freezing point depression by the cytoplasm. It is suggested that osmotic shock modifies a portion of the plasma membrane which interacts with auxin and eventually leads to growth.

Evidence has accumulated recently which suggests that auxin interacts with a membrane as part of its physiological action (6, 18). An auxin effect on the plasma membrane is implied by IAA-induced potential changes (5, 14, 24) as well as the stimulated uptake of K+ (8, 12), Cl" (20), α-aminoisobutyric acid, and 3-0-methyl glucose (Etherton and Rubinstein, in preparation). More direct evidence of an IAA interaction with membranes is based on the specific binding of 3H-IAA to a particulate fraction from corn coleoptiles (11). The identity of this fraction has not yet been reported.

In order to study the nature of the hormone-membrane interaction, coleoptile tissue has been osmotically shocked. In microorganisms, this treatment has been reported to inhibit uptake by releasing components of uptake systems from the membrane (9, 15). Inhibitions of uptake of selected substances after osmotic shock have also been observed in bean leaves (1) and oat coleoptiles (22), and it was suggested that the effect of shock in higher plants may be limited to modifications of the plasma membrane. This report is concerned with how the alteration of the plasma membrane by osmotic shock affects some responses to auxin.

MATERIALS AND METHODS

Coleoptiles were isolated from etiolated seedlings of Avena sativa L. cv. "Garry"; a portion of the epidermis was removed and 5-mm sections were cut as previously described (22). The sections were osmotically shocked by first floating them for 10 min on 0.5 M mannitol and then for 10 min on 1 mM Na-phosphate (pH 6.4) at 4 C.

To measure auxin transport, 1-cm sections were preincubated on Na-phosphate buffer for 30 min. After shocking, a 5-mm segment was cut from the center of each section and placed basal

or apical end down on a 1.5% (w/w) agar receptor block. The sections (four on each receptor) were covered with a donor block containing 10 μM 14C-IAA (52 mCi/mmol) for 90 min after which the tissue and receiver blocks were counted in scintillation fluid.

Growth after 3 hr was determined optically with an ocular micrometer and dissecting microscope. Shorter term growth increments were measured with a position transducer as previously described (4, 19).

The ability of sections to acidify the medium was measured in a manner similar to that of Cleland (2) and Rayle (17). Twenty peeled 5-mm coleoptile sections were placed in 1-dram vials containing 2 ml of 1 mM Na-phosphate (pH 6.45) and a small magnetic stirring bar. After 30 min on a stirrer, the buffer was aspirated and 2 ml of fresh buffer at pH 6.3 added with or without the desired substance. The pH was measured at 20- or 30-min intervals using a Markson "Pencil" electrode and a Beckman Expandomatic pH meter. Standard curves were prepared for each solution by adding known amounts of HCl, thus allowing pH readings to be converted to μequ H+/2 ml.

Cell contents were prepared for determination of concentration of osmoticum by grinding approximately 1 g of peeled coleoptile tissue in a mortar. The supernatant present after centrifugation at 38,000g for 50 min was frozen in Dry Ice containing 95% ethanol. Copper-constantan thermocouples attached to a model 2401 C Hewlet-Packard digital volt meter were used to measure freezing point depression (the plateau reached after supercooling).

RESULTS

The effects of osmotic shock on auxin transport are summarized in Table I. The amounts of auxin transported in both acropetal and basipetal directions were almost identical for the two treatments; furthermore, the transport inhibition by TIBA2 and uptake into the tissue were also similar. Time course curves indicated transport velocities of 6.5 mm/hr for both shock and control treatments (data not shown).

Growth of coleoptile sections was inhibited by osmotic shock when measured 3 hr later (Table II). Length of unshocked controls without IAA increased 3% while auxin-treated sections increased 7%. Osmotic shock inhibited growth of sections both with and without IAA, and the increase seen when IAA was added to shocked sections did not appear to be statistically significant. This experiment was performed on three separate occasions with qualitatively similar results.

To see if auxin-treated, shocked sections were unable to grow merely because of a reduction in turgor, the length of both unshocked and shocked sections was measured after floating on pH 6.5 or pH 5.1 buffers. Other than a slight depression of

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2 Abbreviation: TIBA: 2,3,5-triodobenzoic acid.
growth after shock at both pH values, the data show little differences in growth between shocked and unshocked tissue. Effects on short term growth are presented in Table III. Growth rates of shocked sections were reduced, but both unshocked and shocked treatments showed similar rate increases 7 to 10 min after the addition of a pH 5 solution of Na-phosphate. Acid-stimulated growth was still observed as long as 80 min after shocking. The lag time before the growth rates were accelerated was the same (a few sec) for both treatments at 20 min or 80 min after shock.

The freezing point depression of the cell sap was 1.0 C ± 0.1 C which was an approximate osmoticum concentration of 0.45 m. There were no significant differences between shocked and unshocked tissues 15 to 30 min after treatment.

The effects of osmotic shock on acidification by auxin-treated sections are shown in Figure 1. All treatments led to a small but significant increase in the acidity of the medium for 30 min after cutting, but after 80 min, unshocked sections floated on auxin continued to reduce the pH of phosphate buffer; no changes occurred in the medium around auxin-treated sections which had been osmotically shocked.

During the course of these studies, it was noted that the addition of 1 mm CaCl₂ to the phosphate buffer led to an acidification of this medium by the tissue. The effects of osmotic shock on Ca-stimulated acidification are seen in Figure 2. Sixty min after the addition of 1 mm CaCl₂ to unshocked sections, the rate of acidification was greater than the rate by sections without CaCl₂. A similar trend was observed for shocked treatments. Thus, 60 min after the start of pH determinations, rates of acidification by shocked and unshocked sections in buffer with CaCl₂ were similar, as were the acidification rates of both treatments in plain Na-phosphate buffer.

**Table I.** Effect of Osmotic Shock on IAA Uptake and Transport

<table>
<thead>
<tr>
<th>Direction of Transport</th>
<th>No Shock</th>
<th>Shock</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Uptake</td>
<td>Receptor</td>
</tr>
<tr>
<td></td>
<td>1295</td>
<td>1278</td>
</tr>
<tr>
<td>Apendic</td>
<td>4399</td>
<td>1062</td>
</tr>
<tr>
<td>Basipetal</td>
<td>4899</td>
<td>106</td>
</tr>
<tr>
<td>Total</td>
<td>1348</td>
<td>1278</td>
</tr>
</tbody>
</table>

**Table II.** Effect of Osmotic Shock on IAA-Stimulated Growth of Coleoptile Sections

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Length After 3 Hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>IAA</td>
<td>1.15 ± 0.02</td>
</tr>
<tr>
<td>Shock</td>
<td>1.11 ± 0.02</td>
</tr>
</tbody>
</table>

**Table III.** Effect of Osmotic Shock on Growth Rate of Coleoptile Sections Immediately Before or 7 to 10 Min After Acid Treatment

<table>
<thead>
<tr>
<th>Time of pH Change</th>
<th>0 min After Shock</th>
<th>30 min After Shock</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>4.5</td>
<td>6.0</td>
</tr>
<tr>
<td>No Shock</td>
<td>6.5</td>
<td>5.6</td>
</tr>
<tr>
<td>Shock</td>
<td>6.5</td>
<td>5.6</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Since osmotic shock leads to a rather specific modification of the plasma membrane (1, 15, 22), it can be suggested that part of this modification involves a site of interaction with auxin which eventually controls growth. The inhibition of auxin-stimulated growth by osmotic shock (Table II) is probably not due to a reduced concentration of cell osmoticum and thus turgor. While it is true that ion leakage occurs after shock (22), the tissue is still capable of growing rapidly when the pH is reduced (Table III). The use of acid growth to suggest that the inhibition of the auxin effect is not due to turgor changes, however, is valid only if auxin- and acid-stimulated growth are identical, a possibility with some support (2, 13, 17, but see also 16, 23). Further evidence that osmotic concentration remains unaffected by shock is the failure to find any differences in freezing point depression between shocked and unshocked tissues.

Green and Cummins (7) and Cleland and Rayle (3) have shown that temporary reductions of turgor pressure had no effect on growth rates of coleoptile sections, which seems at odds with data presented here (Table II). The basis for this discrepancy may lie with the concentrations of osmoticum used. Green and Cummins (7) used 1 or 2 bar steps to reduce the pressure of the external medium to ~4 bars; Cleland and Rayle (3) used mannitol concentrations approaching ~5 bars. For osmotic shock treatments, however, the cells were plasmolyzed in a mannitol concentration of ~12.4 bars. Furthermore, restoration to normal turgor occurred in buffers at room temperature (3, 7), but osmotically shocked tissue was rehydrated at 4 C.
which has a more profound effect on membrane transport processes (22).

As stated elsewhere (19), it is impossible to determine precisely what aspects of membrane function are altered by osmotic shock. One possibility is that the inhibition of auxin-stimulated acidification and growth is due to the removal of specific proteins which interact with the hormone; it is also possible that the lowered cell membrane potential, whatever its cause, is the event most closely leading to the prevention of some of the auxin responses.

It is interesting that auxin-stimulated acidification is prevented by osmotic shock (Fig. 1) and this may be the reason that growth by auxin is also inhibited. The acidification caused by CaCl₂, however, continues after shock (Fig. 2). If the two acidification mechanisms were identical, one might conclude that only an auxin-specific portion of the acidification process was modified. It is equally likely, though, that auxin- and CaCl₂-induced acidifications are the result of two different processes (21).

Another interesting finding was the lack of a correlation between auxin-stimulated elongation and polar transport. The suggestion that transport was a requirement for growth (10) has been modified recently by the discovery that auxin transport could be completely inhibited while the auxin-stimulated growth rate was unaffected (25). Here, the inverse situation was shown—auxin-stimulated growth was inhibited while transport remained normal. The use of osmotic shock also leads to the conclusion that the mechanism of polar auxin transport is independent of the acidification mechanism and, possibly, of the cell membrane potential.

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LITERATURE CITED