Abscisic Acid Control of Lectin Accumulation in Wheat Seedlings and Callus Cultures

EFFECTS OF EXOGENOUS ABA AND FLURIDONE

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ABSTRACT

Wheat germ agglutinin is found in wheat embryos and a similar lectin is present in the roots of older plants. We report here that 10 micromolar abscisic acid (ABA) produces an average two to three-fold enhancement in the amount of lectin in the shoot base and the terminal portion of the root system of hydroponically grown wheat seedlings. Although ABA stunts seedling growth, a similar growth inhibition produced by ancyimol is not accompanied by elevated lectin levels. To further clarify the role of ABA, wheat callus cultures were employed. Callus derived from immature embryos was grown on growth medium containing various combinations of ABA and 2,4-dichlorophenoxyacetic acid. Those grown in the presence of 10 micromolar ABA exhibit the largest increases in lectin compared to material grown on other regimes. The involvement of ABA in lectin accumulation was further probed with fluridone, an inhibitor of carotenoid synthesis which has also been linked to depressed levels of endogenous ABA. Wheat seedlings grown in the presence of 10 or 10 milligrams per liter fluridone have few or no carotenoids, and wheat germ agglutinin levels in the shoot base and roots are lower compared to controls. The greatest effect (a 39% reduction in the shoot base) is produced at an herbicide concentration of 10 milligrams per liter. Exogenous 10 micromolar ABA greatly stimulates lectin accumulation in the presence of fluridone, but the levels are not as high as those produced by ABA alone. These results indicate that lectin synthesis is under ABA control in both wheat embryos and adult plants.

Because tissue culture can provide useful information on the hormonal control of development, similar experiments were performed on callus cultures derived from wheat embryos. Recently, lectins have been found in calli and suspension cells of several plants (9, 17, 19). We report that (a) ABA enhances the level of WGA and has marked effects on growth in wheat seedlings, (b) the level of lectin is decreased in the presence of fluridone (2,2-dimethyl-3-phenyl-5-(3-trifluoromethyl)phenyl-4-[1H]pyridinone), an herbicide which reportedly decreases endogenous ABA levels by inhibiting carotenogenesis (2, 11, 24), and (c) the synthesis of lectin is also stimulated by ABA in callus cultures. Some of these results were originally reported by Raikhel et al. (29). Recently, a stimulation of WGA synthesis in wheat roots by ABA was also reported by Stinissen et al. (32).

MATERIALS AND METHODS

Growth of Plants. Wheat grain (Triticum aestivum L. cv Marshall), obtained from the Minnesota Crop Improvement Association, St. Paul, MN, were surface sterilized by alternate 3 min exposures to 70% ethanol, sterile deionized H2O, and 1% NaOCl (diluted commercial bleach) and washed in 3 changes of sterile deionized H2O. The grains were imbibed in aerated deionized H2O for 24 h and then placed on nylon netting supported by a 400 ml beaker containing half-strength Hoagland solution or Peters Hydrosol (Peters Fertilizer Products, Fogelsville, PA). The beaker was placed in a moist plastic chamber which was covered with plastic food wrap to maintain optimal hydration of the grain and seedlings during the first 5 days of growth. The beakers, nylon netting, and media were sterilized by autoclaving, whereas the plastic containers were soaked prior to use in diluted bleach for 5 min and then rinsed exhaustively. 2,4-D was obtained from the Sigma Chemical Co. and added to growth media before autoclaving to produce final concentrations of 2 or 3 mg/L. Stock solutions of ABA (Sigma) were prepared in absolute ethanol and added to sterile media to produce final concentrations of 1 to 100 μM. The final ethanol concentration never exceeded 0.01% and controls contained the same ethanol levels. Fluridone was obtained from Eli Lilly and added directly to media prior to autoclaving to produce final concentrations of 1 or 10 mg/L. Ancyimol (obtained in commercial form as A-rest, Elanco Products, Indianapolis, IN) was used at a final concentration of 1 mg/L. The pH of all solutions was adjusted to 5.5.

Grain were grown in the dark at 25°C or with a 16 h light period at 25°C and an 8 h dark period at 20°C using a programmable incubator or a growth chamber (mixed fluorescent [Sylvania cool-white] and incandescent light; fluence rate 300 μmol m–2 s–1). Solutions were changed every other day to ensure that the levels of active ABA and fluridone remained high during seedling growth. In some experiments, the seedlings were first grown in...
control media for 3, 6, or 9 d and then transferred to media with ABA, fluridone, or ancyclidol.

**Callus Cultures.** Immature embryos, 1.5 to 2.0 mm in length, were obtained from plants 10 to 12 d postanthesis. The embryos were surface sterilized as described above for grain and placed in the dark at 27°C on growth medium consisting of MS inorganic salts, 100 mg/L inositol, 0.4 mg/L thiamine-HCl, 20 g/L sucrose, and 2 mg/L 2,4-D (pH 5.5) (26). Friable, compact callus was usually obtained 20 to 30 d later and was maintained in the dark at 27°C on the same MS medium, except that 3 mg/L 2,4-D was used to prevent rooting. Organ differentiation was induced by transferring callus to media containing altered hormone levels (see "Results") and culturing at 27°C in the dark or light for 20 d.

**Tissue Extraction, Lectin Assays, and Carotenoid Analysis.** To determine lectin levels in seedlings, 8 to 22 plants were assayed at various times as indicated in "Results." The length of the roots and shoots as well as the number of roots were recorded, and then the shoot bases and terminal portion of the root system were each pooled and extracted for WGA as described previously (31). Callus was homogenized and extracted in the same manner, except that the homogenate was further processed in a French pressure cell (American Instrument Co., Silver Springs, MD) at 4.14 × 10⁶ N/m². ELISA were performed as previously described (31). Soluble protein was determined by the method of Bradford (4). For the determination of carotenoids, seedlings were frozen and ground in liquid N₂ and extracted with acetone under dim green light. Absorption spectra were recorded on a DU-7 spectrophotometer (Beckman Instruments).

**RESULTS**

**Effect of ABA on Seedling Growth.** The growth of young wheat plants is severely stunted by 10 μM ABA (Table I), while seedlings grown on ABA at 1 μM are nearly normal in size and grain germinated in 100 μM hormone exhibit almost no growth. These results are consistent with those of Belhanafi and Collet (3) on the growth of young wheat plants. If the medium containing 10 μM ABA is not changed every 2nd d but only replenished with water to compensate for evaporation and transpiration, stunting is less severe, presumably because the effective concentration of ABA is reduced by isomerization and/or metabolic breakdown (reviewed in Milborrow [20]). Our data (Table I) and those of Belhanafi and Collet (3) show that transfer of plants from ABA to control medium releases the inhibition of growth. Plants supplied with 10 μM ABA buffered at pH 6.7 grow faster than those grown at pH 3.9. This result is consistent with the optimal transport of ABA that occurs at low pH (8, 18).

**Enhancement of WGA Levels by ABA.** Despite the reduction in plant growth, the amount of WGA-like lectin detected by ELISA is higher in the shoot base and roots of seedlings grown in 10 μM ABA compared to controls (Table I). Plants grown in 1 μM ABA do not have enhanced lectin levels, whereas those grown in 100 μM ABA are too stunted to assay. Dry wheat embryos contain more than 1 μg of lectin, but the level declines to approximately 100 ng per plant during imbibition, germination, and early seedling growth (21). As previously reported, the amount of lectin fluctuates thereafter in the shoot base of both soil grown plants and those hydroponically maintained on growth medium (31). Throughout the growth course, 10 μM ABA produces an average 2-fold enhancement in the amount of lectin per shoot base, even though the pattern of fluctuations remains the same (Fig. 1). A maximum 3-fold enhancement in the level of lectin is seen in the experiment illustrated in Figure 1 (at d 11), and up to 5-fold enhancement has been observed in other experiments. The same pattern is seen when the data are expressed relative to soluble protein, and values are even higher when based on fresh weight.

When ABA-grown plants are transferred to control medium, the lectin level in the shoot base falls dramatically (Table I). Conversely, lectin is enhanced around 2-fold when seedlings grown in control medium are transferred to 10 μM ABA at d 9 (Fig. 1). Enhanced lectin levels are also detected in seedlings transferred to ABA on d 3 or 6 (data not shown).

Lectin levels are also elevated in the distal part of the root system in seedlings grown continuously in 10 μM ABA (Table I) as well as in those transferred to ABA after an initial period of growth on control medium (data not shown). When seedlings grown continuously in ABA are transferred to control medium, the amount of lectin in the terminal portion of the root system is reduced (Table I).

**Treatment of Seedlings with Ancymidol.** To test whether the ABA enhancement of lectin accumulation is a nonspecific result of growth inhibition, seedlings raised on control medium for 3 d were transferred to medium containing ancyclidol, an inhibitor of gibberellin synthesis (12). At 2 mg/L this agent inhibits shoot growth to the same degree as 10 μM ABA, and root growth is

Table 1. Data from One Experiment on the Effect of 10 μM ABA on Growth and the Level of Lectin in Wheat Plants

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Seedlings</th>
<th>Average Length of Shoots* (cm)</th>
<th>Average Length of Roots* (cm)</th>
<th>Average Number of Roots per Shoot-Base*</th>
<th>Lectin per Shoot-Base (ng)</th>
<th>Lectin in the Distal Portion of the Root System (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth medium for 11 d</td>
<td>19</td>
<td>13.8a</td>
<td>10.2a</td>
<td>9.2a</td>
<td>117.9</td>
<td>35.6</td>
</tr>
<tr>
<td>ABA for 9 d followed by growth medium for 2 d</td>
<td>16</td>
<td>4.0b</td>
<td>5.2b</td>
<td>6.3b</td>
<td>165.6</td>
<td>45.0</td>
</tr>
<tr>
<td>ABA for 11 d</td>
<td>16</td>
<td>2.6c</td>
<td>4.2c</td>
<td>5.8b</td>
<td>222.6</td>
<td>67.1</td>
</tr>
</tbody>
</table>

* Analysis of the means was performed with Duncan's multiple range test (P = 0.05). Letters indicate significantly different values.
ABSCISIC ACID CONTROL OF WHEAT LECTIN ACCUMULATION

- Fluridone at 1 and 10 mg/L fluridone depress lectin levels in the shoot base region and the terminal portion of the root system. The greatest effect is produced at herbicide concentration of 10 mg/L (Table II). The lectin level in the shoot base is depressed 39% and that in the terminal part of the roots is reduced by 17%. As already noted, exogenously applied 10 μM ABA greatly increases the level of lectin in seedlings. In the presence of fluridone, however, the ABA enhancement is reduced, with the greatest effect observed at a fluridone concentration of 10 mg/L. It is noteworthy that the difference between lectin levels in ABA-grown plants and those incubated in ABA plus fluridone is greater than the reduction produced by fluridone alone (compared to controls; Table II).

- Fluridone at 1 mg/L, does not affect seedling growth, although 10 mg/L may depress root growth somewhat (Table II). Thus, the response of wheat seedlings to fluridone is similar to that reported elsewhere (24). On the other hand, the herbicide partially reverses the inhibitory effect of ABA on shoot growth (Table II). ABA-induced stunting of root growth does not respond in the same manner, however. Last, plants continuously grown from germination in the presence of fluridone and ABA in the dark or light are white.

- Experiments with Wheat Callus Cultures. Whole calli obtained from immature embryos were transferred to MS medium containing various combinations of ABA and 2,4-D in the dark or light. The effect of various growth conditions on lectin levels is shown in Table III. Lectin is expressed relative to soluble protein, but a similar pattern is observed when the data are based on final fresh weight. In Table III, the lectin level doubles compared to controls when callus is transferred to the light in the presence of 2,4-D. When 2,4-D is omitted from the medium, the amount of lectin enhancement is 14-fold in the light and 10-fold in the dark. If MS medium is supplemented with ABA and callus is cultured in the light, an 18-fold increase is detected. In contrast, callus grown in the dark in this medium exhibits a 25-fold enhancement of WGA-like lectin. In callus maintained in the light in the presence of both ABA and 2,4-D, the lectin level rises only 14-fold. The value for similar callus grown in the dark is 11-fold. Because of difficulties in initially weighing calli and maintaining sterile conditions, changes in fresh weight under various growth regimes were not determined. However, many thin roots appeared on all calli with elevated lectin levels.

- Calli were also obtained from root tips and shoot bases of wheat seedlings. These calli exhibit similar patterns of root growth and elevated ABA levels when subjected to inductive conditions.
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Table II. An Experiment on the Effects of (10 μM) ABA and Fluridone on Growth and the Level of Lectin in Wheat Plants

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Seedlings</th>
<th>Average Length of Shoots*</th>
<th>Average Length of Roots*</th>
<th>Lectin per Shoot Base</th>
<th>Lectin in the Distal Portion of the Root System</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>cm</td>
<td>ng</td>
<td>per plant</td>
<td></td>
</tr>
<tr>
<td>1. Control growth medium</td>
<td>14</td>
<td>17.3a</td>
<td>13.1a</td>
<td>142.5</td>
<td>31.6</td>
</tr>
<tr>
<td>2. Fluridone, 1 mg/L</td>
<td>16</td>
<td>16.9a</td>
<td>13.0a</td>
<td>136.3</td>
<td>28.9</td>
</tr>
<tr>
<td>3. Fluridone, 10 mg/L</td>
<td>11</td>
<td>17.2a</td>
<td>11.0b</td>
<td>85.6</td>
<td>26.3</td>
</tr>
<tr>
<td>4. ABA</td>
<td>13</td>
<td>7.8c</td>
<td>8.2c</td>
<td>261.8</td>
<td>58.3</td>
</tr>
<tr>
<td>5. ABA and fluridone (1 mg/L)</td>
<td>13</td>
<td>10.1b</td>
<td>6.6d</td>
<td>228.4</td>
<td>43.4</td>
</tr>
<tr>
<td>6. ABA and fluridone (10 mg/L)</td>
<td>22</td>
<td>11.1b</td>
<td>6.5d</td>
<td>187.8</td>
<td>31.9</td>
</tr>
</tbody>
</table>

*Analysis of the means was performed with Duncan’s multiple range test (P = 0.05). Letters indicate significantly different values.

Table III. Lectin Levels in Callus Cultures Derived from Immature Embryos

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lectin Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ng per mg</td>
</tr>
<tr>
<td></td>
<td>soluble protein</td>
</tr>
<tr>
<td>MS and 2,4-D (dark)*</td>
<td>4.8</td>
</tr>
<tr>
<td>MS (dark)*</td>
<td>48.6</td>
</tr>
<tr>
<td>MS and ABA (dark)*</td>
<td>119.1</td>
</tr>
<tr>
<td>MS, ABA and 2,4-D (dark)*</td>
<td>53.9</td>
</tr>
<tr>
<td>MS and 2,4-D (light)*</td>
<td>11.1</td>
</tr>
<tr>
<td>MS (light)*</td>
<td>66.5</td>
</tr>
<tr>
<td>MS and ABA (light)*</td>
<td>86.3</td>
</tr>
<tr>
<td>MS, ABA and 2,4-D (light)*</td>
<td>65.9</td>
</tr>
</tbody>
</table>

*Control callus lacks roots.  b Callus subjected to inductive treatment has roots.

DISCUSSION

Our results show that ABA strongly influences the level of lectin that accumulates in the emerging roots of wheat plants. Previous work in our laboratory has established that this protein is a lectin with biochemical and immunological properties similar if not identical to those of embryo WGA (31). These results have recently been confirmed by Stinissen et al. (32). It has been shown that ABA stimulates the formation of several proteins in developing wheat grain including scutellar storage proteins and WGA (33). ABA exerts similar effects on the seeds of several other species as well (6, 7, 10, 27). In the present study, and as reported previously (29), we have found that the amount of lectin in the roots of wheat plants is substantially elevated by exogenous 10 μM ABA. Similar results were recently obtained elsewhere (32). This increase does not appear to be a nonspecific effect of the growth retardation produced by ABA, since a similar elevation does not accompany application of ancydrol, a growth inhibitor which interferes with gibberellin action (12). Furthermore, lectin accumulation is also stimulating by ABA in callus cultures which normally have low levels of this protein. In plants treated with fluridone, a herbicide thought to inhibit endogenous ABA formation by interfering with the carotenoid biosynthesis pathway, lectin levels are reduced. This effect is reversed by exogenously supplied hormone.

We believe that the elevation in lectin level is brought about by a specific effect of ABA on roots. Our previous studies show that there is a direct relationship between lectin and the presence of roots. Lectin is preferentially localized in root tips, including the cap (22, 30). Furthermore, the highest levels are associated with new roots that form at the base of the shoot, and less is present in older roots (21). Immunocytochemistry reveals that lectin is found on root primordia even before they emerge from the shoot base (22). In callus cultures, treatments that lead to the formation of new roots (such as light and the omission of 2,4-D) in the absence of exogenous ABA raise lectin levels (omission of 2,4-D also increases the amount of lectin in tissue cultures of Dolichos biflorus; 17). The addition of ABA boosts these levels even further. Since our data (e.g. Table I) and those of others (24) show that ABA doesn’t increase root number or growth, the enhancement of lectin level probably results from a direct effect on those roots or root primordia present before ABA treatment began or formed during application of the hormone. Thus, the expression of normal lectin levels may be controlled by endogenous hormone that is formed in new roots during organogenesis. This would explain the increase in lectin during root formation in callus cultures in the absence of exogenous ABA. Inclusion of this hormone in the medium then increases lectin levels even further. It is worth noting in this regard that treatments used to enhance rooting, (i.e. light and removal of auxin) may also stimulate endogenous ABA synthesis (1). The effect of ABA on the induction of seed-specific proteins appears to be mediated at least in part by an increase in the level of certain mRNAs (7, 14-16, 25, 28). It is reasonable to speculate, therefore, that ABA has a similar effect during the enhancement of lectin accumulation in roots.

Our results with fluridone are compatible with previous information on this herbicide (2, 11, 24). Seedlings grown under the influence of this agent, although similar in size to control plants, are white, indicating a lack of carotenoids. Spectrophotometric analysis of acetone extracts confirm that these pigments are absent in fluridone-treated seedlings. Previous experiments with fluridone and norflurazon, another herbicide that inhibits carotenogenesis, indicate that endogenous ABA levels are also affected (13, 24). The lack of endogenous ABA is presumably responsible for the induction of vivipary observed after herbicide application (11). Our work shows that lectin levels decline under the influence of fluridone, which would be expected if ABA synthesis is inhibited. Furthermore, this effect is reversed by 10 μM ABA, which also stunts growth as it does in the absence of herbicide. However, since (a) lectin accumulation is not com-
pletely eliminated by fluridone and (b) the lower concentration (1 mg/L) has less of an effect even though the loss of carotenoids is nearly as complete as with 10 mg/L, the relationship between fluridone, ABA, and lecint levels is not clear cut. This conclusion is supported by other data in Table II noted earlier.

Explanations for these results are not apparent. It is possible that WGA levels are reduced due to nonspecific or toxic effects of fluridone, rather than to an inhibition of ABA biosynthesis. This might account for the lower lecint levels in plants grown in ABA plus fluridone compared to ABA alone. It is also possible that the fluridone effect on ABA biosynthesis is complex. Fluridone is thought to inhibit carotenoid formation near the phytoene-phytofluene level (2), but a definitive determination has not been reported. Furthermore, the formation of ABA relative to the mevalonic acid-carotenoid pathway is not well understood, and debate centers on a pathway versus a biosynthetic route closer to mevalonic acid (20). Our results support an effect of the herbicide on hormone levels, but a more definitive picture is still not available.

Continued progress in our understanding of the relationship between ABA, organogenesis and lectin synthesis, as well as the mechanism of fluridone action, will depend on new data on endogenous ABA levels. Although a number of studies indicate that ABA is present in root tips where it could control growth and gravitropism (1), other experimental data contradict this conclusion (24). We are employing immunological assays to quantitate ABA levels in material treated with fluridone and/or exogenous hormone. We hope to ascertain whether changes in lectin that occur during normal growth (31) and in response to applied agents are correlated with alterations in endogenous hormone levels.

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