Effects of Light on Protein Patterns in Gravitropically Stimulated Root Caps of Corn

LEWIS J. FELDMAN* AND Viqui GILDOW
Department of Botany, University of California, Berkeley, California 94720

ABSTRACT

In certain cultivars of corn (Zea mays var. Merit), light stimulates gravitropic bending of the root by influencing events in the root cap. In this paper, we report on changes in root cap proteins which occur as a result of the light treatment and single out specific proteins as potentially having a role in mediating the gravitropic response. For this work, we have used root caps maintained aseptically in culture media supplemented with auxin. If auxin is deleted from the culture medium, the protein profiles observed following illumination differ from that seen in caps provided light while in auxin-supplemented media. We also report that several of the proteins for which synthesis is stimulated by light appear to turn over rapidly, usually within 0.5 hour of formation.

In some cultivars of corn, light is able to initiate gravitropic bending of seedling roots (downward growth) by affecting processes in the root cap (4, 16). If seedlings of these cultivars are maintained in continuous darkness or in dim green light, roots fail to respond to gravity and instead grow in a direction determined by the orientation of the seedling. Recently we have shown that exposure of the cap to white light leads not only to root bending but also stimulates protein synthesis within the cap, with protein levels enhanced 1.5 to 2 times that observed in caps maintained in complete darkness (5, 6).

Protein synthesis is necessary for gravitropic bending (5). In cultured caps in which protein synthesis is inhibited prior to exposure to light, processes leading to downward root bending are interrupted. Substituting these cultured caps in place of caps from dark-grown roots does not elicit any root bending, even if these roots are exposed for considerable periods (in excess of 1 h) to continuous white light (5).

In this paper, we define more precisely the role of protein synthesis in the processing of the gravity stimulus into a growth response. In particular, we examine whether the light-induced gravity translation mechanism involves: (a) the synthesis of one or more unique proteins; or (b) a general enhancement in the levels of many proteins; or (c) the enhancement of the levels of selected proteins. In order to distinguish between these possibilities, we have employed for some of this work a double radioisotope-labeling technique. Briefly, this technique involves labeling one lot of tissue with one isotope (3H) under one set of experimental conditions and a second lot of tissue with a different isotope (35S) under some other experimental conditions. Labeled tissues from both treatments are then combined and the proteins purified as one lot. Following purification, the protein-labeling patterns are examined either by one- or by two-dimensional gel electrophoresis. Through the use of one-dimensional gel electrophoresis, the ratio of label (3H/35S) is obtained and is plotted as a function of position along the gel. Differences in the ratio, compared to the general background, are indicative of changes in either the rate of synthesis or turnover of particular proteins. Two-dimensional gel electrophoresis is coupled with an autoradiographic technique and is designed to allow one to distinguish between the various radioisotopes through the use of photographic films of varying sensitivities. In theory, this technique allows one to visualize protein patterns two-dimensionally and to compare the patterns obtained under different experimental conditions.

MATERIALS AND METHODS

Chemicals and Radioisotopes. [35S]Methionine (1010 Ci/mmol) and [3H]methionine (86 Ci/mmol) were obtained from Amersham. All electrophoresis chemicals were obtained from Bio-Rad. NP40, pepstatin A, and leupeptin were obtained from Sigma. All other chemicals were standard reagent laboratory chemicals.

Plant Material and Irradiation. Grains of Zea mays var. Merit (Asgrow Seed Co., MI) were grown aseptically as described previously (4). For labeling experiments, 150 to 200 caps were excised in dim green light (515–545 nm), with the aid of a dissecting microscope and fine dissecting tools.

Radioactive Labeling of Proteins in Root Caps. Following excision, caps were transferred aseptically in green light to 0.2 ml of S2M medium (±IAA) (4, 5), and allowed to equilibrate 1 h in the dark at 25°C on a shaker (100 rpm). Following equilibration, the label was added in green light to yield the following concentrations: [3H]methionine, 80 μCi/ml; [35S]methionine, 130 μCi/ml. The tissues were returned to the shaker in the dark and allowed to equilibrate for an additional hour. At the end of the second equilibration period, tissues were either maintained in the dark on the shaker (dark controls, ±IAA) or the tissue (±IAA) was illuminated for 10 min with white light (1.3 × 10−2 mol m−2 s−1), and then returned to the dark. At varying intervals (0.5–5 h) after return to the dark lots of caps were extracted for protein and the proteins purified for one- and two-dimensional polyacrylamide gel electrophoresis. For double label experiments, caps were incubated in either [3H] or [35S]methionine according to the schedule outlined in Table I.

Preparation of Labeled Protein for Polyacrylamide Gel Electrophoresis. At the end of the various incubation periods, the tissues from the corresponding double label experiments were pooled and homogenized in 2 to 3 ml of 50 mM Tris-HCl (pH 7.4), 5 mM MgCl2, 10 mM urea, 1 mM EDTA, 2% (v/v) mercaptoethanol, and 500 mM (NH4)2SO4. In addition, for some of the double labeled tissues, the protease inhibitors pepstatin A...
and leupeptin were included in the homogenization medium at a concentration of 1 and 0.1 mM, respectively (1). Following homogenization, the tissue was spun for 30 min at 10,000g, and the supernatant was removed and enough (50%) (w/v) TCA was added to yield a final concentration of 10% TCA. The proteins were allowed to precipitate for 2 h on ice and then collected by spinning for 30 min at 10,000g. The supernatant was decanted and the protein pellet washed several times with cold 80% acetone followed by 2 washes with 100% cold acetone. For one-dimensional gel electrophoresis, the precipitate was solubilized in 62.5 mM Tris, pH 6.8, 10% (w/v) glycerol, 3% SDS, 5% (v/v) mercaptoethanol. Following solubilization, the proteins were loaded onto gels. One-dimensional gel electrophoresis was carried out in the presence of SDS on a 1-mm thick gradient (10–15%, w/v) slab gel after the method of Laemmli (12). Two-dimensional gel electrophoresis with isoelectric focusing as the first dimension was performed essentially according to the methods of O’Farrell (14) on 0.3 x 10 cm tubes. The protein sample was dissolved in 9.5 M urea, 2% (w/v) NP40, 5% (v/v) mercaptoethanol, 1.6% (v/v) pH 5 to 7 Ampholines, 0.4% (v/v) pH 3.5 to 10 Ampholines, applied to the top of the gel, overlaid with 8.0 M urea, 5% (w/v) NP40, 0.8% (v/v) pH 5 to 7 Ampholines, 0.2% (v/v) pH 3.5 to 10 Ampholines, and focused for a total of 6100 v-h (12 h at 400 v, 1 h at 800 v plus 0.5 h at 1000 v). At the end of the focusing period the gels were removed from the tubes, equilibrated for 2 h on a shaker in 10% (w/v) glycerol, 5% (v/v) mercaptoethanol, 2.3% (w/v) SDS, 62.5 mM Tris, pH 6.8, and then each gel was applied individually to the top of a polyacrylamide gradient gel (as described previously). At the end of this second electrophoretic run, gels were fixed and stained. For proteins run in one dimension only, each lane of the gel, for both single and double label experiments, was sliced into 0.75-mm segments, beginning at the top of each lane, and each slice individually placed onto the bottom of a scintillation vial. To each vial, 0.5 ml of 30% H2O2 was added and then the vials were sealed tightly and placed into a 50°C oven for 18 h. After allowing the vials to cool to room temperature, 7 ml of PCS Scintillation Fluid (Amersham) were added. Counting was done on a Hewlett Packard scintillation counter. For single label experiments with 3H, the channel was set at 20 to infinity. For double label experiments, the 3H channel was set at 20 to 350 and the 35S channel set at 700 to infinity. At this setting, approximately 2 to 4% of the 3H counts were detected by the 35S window and about 15% of the 35S counts were recorded by the 3H window.

Fluorography. Fluorography was performed according to the methods of McConkey (13). This method involves two steps: autoradiography using sequentially Kodak XR5 and NO-screen x-ray films.

Table I. Double Label Experiment Treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>35S</th>
<th>3H</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No light</td>
<td>+Light</td>
</tr>
<tr>
<td></td>
<td>+IAA</td>
<td>+IAA</td>
</tr>
<tr>
<td></td>
<td>+0.5 h dark</td>
<td>+0.5 h dark</td>
</tr>
<tr>
<td>2</td>
<td>+Light</td>
<td>+Light</td>
</tr>
<tr>
<td></td>
<td>Minus IAA</td>
<td>+IAA</td>
</tr>
<tr>
<td></td>
<td>+0.5 h dark</td>
<td>+0.5 h dark</td>
</tr>
<tr>
<td>3</td>
<td>+Light</td>
<td>+Light</td>
</tr>
<tr>
<td></td>
<td>+IAA</td>
<td>+IAA</td>
</tr>
<tr>
<td></td>
<td>+5 h dark</td>
<td>+0.5 h dark</td>
</tr>
<tr>
<td>Control</td>
<td>Dark</td>
<td>Dark</td>
</tr>
<tr>
<td></td>
<td>+IAA</td>
<td>+IAA</td>
</tr>
</tbody>
</table>

Fig. 1. 3H count profiles for proteins extracted from root caps which were illuminated for 10 min and then returned to the dark for the period indicated prior to extracting the proteins. The proteins were separated on polyacrylamide gels and the gels subsequently cut into 0.75-mm slices.

RESULTS

Single Label Experiments. Cap tissues incubated in medium supplemented with [3H]methionine incorporated label into numerous proteins (Fig. 1) with a majority of the label associated with proteins in the 23- to 80-kD range.

If cap tissue was illuminated with white light for 10 min, returned to the dark, and after varying periods in the dark the distribution of 3H in protein was examined, a fairly consistent
Fig. 2. Effects of light, auxin, and increasing periods of darkness on protein profiles from root caps as measured by double labeling with \(^{3}H\) and \(^{35}S\). Double labeling was carried out as indicated in the text and the proteins run on polyacrylamide gels, the gels then cut into 0.75-mm slices, and the amount of \(^{3}H\) and \(^{35}S\) measured. The ratios for the \(^{3}H\) to \(^{35}S\) were obtained and plotted as a function of position on the gel.

A pattern in incorporated label was observed for all tissues except 0.5 h. After 0.5 h in the dark, a relatively large amount of \(^{3}H\) was associated with a low molecular weight protein(s) located 90 mm (out of a possible 115 mm) from the top of the gradient gel. Proteins in this region of the gel at 0 h and at all other time points did not show this high level of \(^{3}H\) incorporation. Light also stimulated an increase in the relative sizes of several other peaks (e.g., the peak in the 5-h treatment located 70 mm from the top of the gradient gel). These changes, however, represented no more than a doubling in relative peak height when compared to corresponding peaks from caps maintained continuously in the dark. There was no evidence that light repressed the synthesis of any protein or protein fraction.

One-Dimensional Electrophoretic Analysis of Double Labeled Proteins. A \(^{3}H\) to \(^{35}S\) ratio was obtained for each of the double label experiments in Table I. For tissues maintained in the light (\(^{3}H\)-labeled) versus dark (\(^{35}S\)-labeled), the light treatments enhanced above background the \(^{3}H\) label in three regions of the gel (53, 72, and 90 mm from the top of the gradient gel) (Fig. 2). In illuminated tissues provided IAA (\(^{3}H\)-labeled) versus tissues deprived of auxin (\(^{35}S\)-labeled), two peaks (at 70 and 94 mm from the top of the gradient gel) showed an increase in the ratio of \(^{3}H\) to \(^{35}S\). The ratio pattern in label from tissues provided light followed by either 0.5 h dark (\(^{3}H\)-labeled) versus 5 h darkness (\(^{35}S\)-labeled) shows three peaks (at 52, 72, and 91 mm from the top of the gradient gel) at which there are selective increases in \(^{3}H\) incorporation.

Double Label Experiments Coupled with Two-Dimensional Gel Electrophoresis. Proteins from double label experiments were examined by the method of two-dimensional gel electrophoresis coupled with sequential exposure of the gels to x-ray films of varying sensitivities. For the double label experiments, three different treatments, all \(^{35}S\)-labeled, were individually com-
pared to the $^3$H-labeled tissues (Table 1). For each of the double label experiments, the distribution of labeled proteins was recorded by two types of x-ray film: one sensitive to both $^3$H and $^{35}$S and the other sensitive to $^{35}$S only. By comparing the two exposed films, our intent was to distinguish whether proteins from the various $^{35}$S-labeled tissues differed in their labeling pattern from the $^3$H-labeled caps. Although we were able to resolve over 150 individual polypeptides, we were unable to demonstrate any qualitative differences in the protein patterns in any of the three treatments. However, by comparing the fluorographs to the labeled gels, we observed that a number of proteins which stained with Coomassie blue remained unlabeled, and that some proteins which were heavily labeled were barely detectable, if at all, with the Coomassie stain.

**DISCUSSION**

Roots of *Zea mays* var. Merit require protein synthesis in the root cap for the translation of the gravity stimulus into a growth response (5). From previous tissue culture work, we have shown that both light and auxin are required in this response. To date, relatively little is known of the physical and biochemical processes in the cap associated with gravitropic bending in roots. One of the favored views is that growth inhibitors originate in the cap in response to a gravity stimulus (and in some roots in response to light as well) and from the cap move to the root proper where downward bending is promoted (15). A number of investigators have shown that light treatments can modify and enhance the gravitropic response (3, 7, 10), but the mechanism by which this modification occurs about is not known. Suzuki et al. (17) have suggested that a possible regulatory effect of light is in modifying the NADP/NADPH levels in the root leading to downward bending. From the work with cress roots, Hart and Macdonald (9) provide data for the involvement of the two photosystems in the light-mediated gravity response. For coleoptiles, Hild (11) has suggested that the transduction of gravity and light stimuli must share some steps common to both pathways. The nature of the linkage between the two tropisms, however, as yet remains obscure.

Because our earlier work had shown that light stimulates protein synthesis in the cap and that this synthesis is necessary for downward growth of the root, we proposed (6) that one of the ways in which light, in the presence of auxin, mediated the gravity response was by stimulating the *de novo* synthesis preferentially of one or more proteins associated with the gravitropic mechanism.

The labeled protein profiles obtained from tissues maintained either continuously in the dark or given light are remarkably similar with no apparent qualitative differences (Fig. 1). However, to order to more fully address the question of possible qualitative differences, further analysis was accomplished with two-dimensional electrophoresis combined with a double label. Using this technique, we were unable to demonstrate any qualitative differences in protein profiles from either light versus dark or plus auxin versus minus auxin (Fig. 3). We therefore have concluded that light in combination with auxin leads to an enhancement in the synthesis of specific pre-existing proteins, rather than preferentially stimulating the *de novo* formation of selected proteins.

One such pre-existing protein(s) with marked light-enhanced synthesis was located 90 mm from the top of the gradient gel (Fig. 1, 0.5 h). Since this increase in label at 90 mm was observed only after 0.5 h in darkness and was undetectable in tissues maintained in the dark for longer periods (1–5 h), we would conclude that the protein(s) at 90 mm is short-lived and that in whatever manner light acts, its effect on the synthesis of this protein is of a transitory nature. Of interest is the fact that the appearance and disappearance of label in this protein correlate temporally with the formation and subsequent disappearance from the root cap of light-inducible inhibitory growth substances (4). The short-lived nature of this protein is reminiscent of several animal proteins such as ornithine carboxylase with a half-life of 0.2 h and 5-aminolevulate synthetase with a reported half-life of 0.34 to 1.1 h (8). Goldberg and St. John (8) suggest that rapid degradation of certain proteins evolved in order that their intracellular levels could respond rapidly to various environmental stimuli. Viewed in this context, the rapid degradation and synthesis of certain root cap proteins may be important in adapting the root to particular environmental, such as light or dark.

In the double label experiments coupled with one-dimensional gel electrophoresis (Fig. 2), we compared the protein profiles of three experimental treatments (all $^{35}$S-labeled) with profiles of $^3$H-labeled proteins obtained from caps provided auxin plus 10-min light, followed by 0.5 h darkness. By comparing the profiles of the $^3$H/$^{35}$S ratio of $^3$H/$^{35}$S labeled tissues. For light versus dark, the increases pointed to proteins preferentially enhanced in their synthesis by the light treatment. The peaks observed in the light versus dark were also observed in the 0.5 versus 5 h experiment, but here the increased ratios were not only indicative of that protein synthesis induced by light, but, moreover, suggest that some of these proteins turn over within the 5-h dark period. Finally, for the plus versus minus auxin treatments, an increase in the $^3$H/$^{35}$S ratio was observed mainly in the proteins 70 mm from the top of the gel with a slight increase in the ratio at 90 mm. Also observed was a reduction (compared to the general background) in the label ratio 82 mm from the top of the gel. These selective increases in the ratio suggest that auxin can preferentially affect the synthesis and/or turnover of particular proteins. Such a stimulatory role for auxin is consistent with the numerous reports noting its effect on protein synthesis (2). Of interest is the region of the protein profile showing a relative decrease in the $^3$H/$^{35}$S ratio, suggesting that in the presence of auxin the synthesis of certain proteins is either retarded or that the turnover of specific proteins is enhanced.

From this work, we would conclude that light, in part, affects gravitropic root bending by enhancing the levels of selective, pre-existing proteins and that in the dark these proteins turn over rapidly to some level. There was no evidence for light-stimulated *de novo* protein synthesis.

**LITERATURE CITED**

2. BATES GW, RE CLELAND 1980 Protein patterns in oat coleoptiles as influenced by auxin and protein turnover. Planta 154: 189-192