Communication

Gibberellic Acid Effects on Greening in Pea Seedlings

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ABSTRACT

The effect of gibberellic acid (GA) on light-induced greening of etiolated pea plants (Pisum sativum [L.] cvs Progress and Alaska) was characterized. Progress, a GA-deficient dwarf of Alaska, was found to accumulate chlorophyll and light harvesting chlorophyll protein associated with photosystem II (LHC-II) more rapidly than Alaska, Alaska treated with GA, or Progress treated with GA. A slightly lower chlorophyll content was noted after 24 hours of light induced greening for Alaska treated with GA relative to untreated Alaska. GA-treated Progress, Alaska, and GA-treated Alaska all gave essentially identical patterns for LHC-II accumulation. Similar patterns of LHC-II mRNA induction were found in all four treatments indicating that differences in mRNA induction did not cause differences in LHC-II accumulation. Chlorophyll and LHC-II accumulation in each treatment followed the same patterns of accumulation and a significant correlation (at the 0.01 level of significance) was found between chlorophyll and LHC-II content. Since Progress treated with GA accumulated LHC-II and chlorophyll in a manner similar to that of Alaska, it is clear that GA alters the process of greening either directly or indirectly.

The LHC-II complex is an integral component of the antenna Chl complexes in the thylakoid membranes of higher plants (26) and is coded by a nuclear, multigene family (6, 8, 21). LHC-II genes are regulated by light (4, 11, 25) through the phytochrome system. The biosynthetic pathway for Chl similarly depends on phytochrome activation (1). Chl has been implicated to be a limiting factor in the formation of LHC-II complexes (15, 17, 18). Recently using light intensity as a means of varying the rate of Chl accumulation it was shown that LHC-II accumulation mimics Chl accumulation (18). Other investigators have also shown LHC-II accumulation mimicking Chl accumulation (2, 3, 12). In this report two pea cultivars, Alaska and Progress (a GA-deficient dwarf of Alaska), are shown to green at different rates. Previously, addition of exogenous GA has been shown to cause changes in populations of translatable mRNAs and accumulated polypeptides in dwarfs of maize and pea (7). The tall/dwarf phenotype in Progress pea is controlled by a single Mendelian gene designated le and acts during a single enzymatic step in GA biosynthesis (22). Addition of exogenous GA to cv Progress has been shown to produce internode lengths similar to the GA-sufficient cv Alaska. Exogenous GA was applied to Progress and Alaska pea cultivars to determine its effect on greening. Chl content, LHC-II content, and LHC-II mRNA induction were determined for etiolated pea seedlings greened for a 24-h period.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds for Pisum sativum [L.] cvs Progress and Alaska were germinated in moist vermiculite at 25°C in the dark for 7 d. The manipulation of the peas in the dark was done using a green safelight constructed from a combination of 2.75-W tungsten illuminator and green filter (Bausch and Lomb 31-35-61). The etiolated plants were placed in continuous white light at 27°C. The light was from fluorescent and incandescent lamps that produced an intensity of 275 to 300 μmol·m⁻²·s⁻¹ at the top of the pea seedlings. GA obtained from Carolina Biological Supply was applied each day after planting to one set of Alaska and Progress plants. The GA was applied as a spray in a concentration of 2 mM. Three separate sets of plants were germinated and greened in this manner. The internode lengths were measured on five seedlings from each treatment to check for a similar GA effect during each of the greening experiments.

Chl determination

Chl was extracted from pea leaves with DMF. Chl content was measured as described by Moran (19). Three separate greening experiments were conducted and Chl accumulation measured. Chl a/b ratios were also determined spectrophotometrically by the methods of Moran (19).

Northern Blot Analysis

The plasmid pAB96 containing cloned LHC-II cDNA from pea (4) was the gift of N.-H. Chua (Rockefeller University, New York). The 800-nucleotide insert from pAB96 was isolated as described previously (17) and used as probe for LHC-II mRNA. Total RNA was isolated from the leaves of Progress and Alaska pea plants and from the leaves of Progress and Alaska pea plants treated with GA. RNA was isolated from etiolated peas and peas greened for 2, 4, or 24 h for all treatments. RNA was isolated for each treatment during two separate greening experiments. One additional greening experiment involved RNA isolation from etiolated Progress and Alaska peas and from those illuminated for 2, 4, 10, 24, and
The methods used for isolation of total RNA were those of Mathis and Burkey (17). Samples of total RNA (10 μg) from pea leaves were separated on 1.8% agarose gels containing 6% formaldehyde and 1 X Mops buffer (20 mM Mops, 5 mM sodium acetate, 1 mM EDTA [pH7.0]) (17). Transfer of RNA to Gene Screen filter membranes and hybridization of labeled LHC-II cDNA to total RNA was performed as described previously (17). Filters were first washed in 1 X SSC (0.15 M NaCl, 0.015 M sodium citrate), 0.1% SDS at room temperature (2 times for 15 min each time) and at 65°C (2 times for 20 min each time). A second wash was performed in 0.1 X SSC, 0.1% SDS (2 times for 15 min each time) at 65°C. Filters were then wrapped in plastic wrap and autoradiography conducted at −80°C using Kodak XAR5 film in Kodak X-Omatic cassettes with intensifying screens.

Single Radial Immunodiffusion Analysis

Antibodies for LHC-II polypeptides were prepared previously (17). Single radial immunodiffusion analyses were performed by methods described by Mathis and Burkey (17). Purified pea LHC-II was used as a standard. Protein extracts were prepared from the leaves of pea plants during two separate greening experiments for Progress and Alaska and each cultivar treated with GA. LHC-II content from the leaves of etiolated seedlings and seedlings greened for 2, 4, 6, and 24 h was measured for all treatments except cv Alaska treated with GA. For cv Alaska treated with GA, etiolated seedlings and plants illuminated for 4 and 24 h were tested for LHC-II content. Regression analyses and correlation coefficients for Chl accumulation versus LHC-II accumulation were determined by standard statistical methods (24).

RESULTS

Plant Height and Morphology of Pea Buds

In three separate greening experiments, 7 d old etiolated pea seedlings had mean plant heights of 14 cm ± 1 cm for cv Progress, 21 cm ± 1 cm for cv Alaska, 21 cm ± 1 cm for cv Progress with GA added, and 22 cm ± 1 cm for cv Alaska with GA added. For each cultivar and cultivar plus GA combination, three internodes had developed during 7 d growth in the absence of light. During the 24 h of greening, plant-height increases were less than 1 cm. Leaves on the tops of Progress-plus-GA plants were morphologically similar to those on Alaska and Alaska-plus-GA plants. Leaves on the tops of Progress plants were larger than those found in the other three treatments.

LHC-II mRNA Accumulation

The induction and accumulation of LHC-II mRNA in the leaves of pea cultivars Alaska and Progress were not affected by addition of GA (Fig. 1). LHC-II mRNA levels increased during the first 4 h of greening relative to the levels present in etiolated tissue for cvs Progress and Alaska and each cv treated with GA. In a separate greening experiment where pea cultivars Alaska and Progress were greened for 2, 4, 10, 24, and 48 h similar patterns of induction and accumulation were also observed (data not shown). The low levels of LHC-II mRNA present prior to exposure to white light may have been induced by the green safelight. After 4 h of light-induced greening, cv Progress and cv Progress treated with GA had approximately twofold more LHC-II mRNA than either cv Alaska or cv Alaska treated with GA (as determined by densitometry of autoradiographs). In cv Progress and cv Progress treated with GA, the level of LHC-II mRNA was similar after 4 and 24 h of greening. In cv Alaska and cv Alaska treated with GA the quantity of LHC-II mRNA increased twofold between 4 and 24 h of light-induced greening.

LHC-II and Chl Accumulation

During the first 24 h of light-induced greening, cv Progress accumulated significantly more LHC-II and Chl per gram of leaf fresh weight than did cv Alaska (Fig. 2). However, cv Progress treated with GA gave LHC-II and Chl accumulation patterns very similar to those of cv Alaska. A slightly lower Chl content was noted for cv Alaska treated with GA relative to cv Alaska after 24 h of greening (Fig. 2). Chl a/b ratios were inversely proportional to the quantity of LHC-II in each treatment (Table I) as demonstrated previously by Leong and Anderson (16). Higher Chl a/b ratios were demonstrated for pea cv Alaska with or without GA treatment and for cv Progress treated with GA than for cv Progress after 4 or 24 h of light induced greening. The data presented in Table I for Progress peas are similar to those collected by Burkey (5) when Progress peas were greened under similar conditions and indicate that Chl b content increases with LHC-II content. In cvs Progress and Alaska Chl a/b ratios stabilized after 24 h of greening, since these values were identical for plants greened either 24 or 48 h.
The differences in LHC-II and Chl content in Figure 2 were determined on a per gram fresh weight of tissue basis. If they were normalized to a per plant or per leaf basis, the elevated levels of LHC-II and Chl in cv Progress would be accentuated when compared to cv Alaska with and without GA treatment or cv Progress treated with GA since the leaves on Alaska, Alaska-plus-GA, and Progress-plus-GA plants are smaller. A significant correlation (significant at the 0.01 level) between Chl and LHC-II content was noted during light-induced greening for each treatment (data not shown). A similar correlation had previously been shown for pea and barley plants when light intensity was used to vary the rate of Chl accumulation (18).

**DISCUSSION**

Similar inductions of mRNA occurred in cvs Progress and Alaska and in each cv treated with GA. A lack of coordination between LHC-II mRNA and LHC-II protein accumulation was noted when comparing cv Progress with and without GA treatment (Figs. 1 and 2). This lack of coordination indicates that mRNA did not limit LHC-II accumulation. These data are in close agreement with those obtained previously (13, 17, 18). In this study our data may also indicate that in Alaska pea plants Chl availability limits LHC-II accumulation when compared to Progress pea plants. Klein et al. (15) have recently indicated that Chl is essential to the accumulation of LHC-II polypeptides. They concluded that absence of Chl results in turnover of LHC-II polypeptides in the presence of LHC-II mRNA. Other investigators have also indicated that Chl content reflects LHC-II content (2, 3, 12). These data collectively suggest that Chl accumulation may limit LHC-II accumulation.

GA clearly alters both Chl and LHC-II accumulation patterns in Progress peas. Perez et al. (20) have shown that a mutant of tomato with increased levels of GA$_3$ contains less Chl. In this present study it was observed that exogenously applied GA slowed Chl and LHC-II accumulation rates in cv Progress (a GA-dwarf of cv Alaska) to rates indistinguishable from cv Alaska. It is plausible that this effect may be through an interaction with phytochrome, since both LHC-II and Chl are influenced by phytochrome. Moreover, other investigators have recently shown that addition of GA$_3$ in *Kalanchoë* (23) influences the rate of phytochrome-mediated seed germination.

From our data the specific influence of GA on LHC-II mRNA in pea appears to be negligible. However, in *Arabidopsis thaliana*, GA$_3$-germinated etiolated seedlings contained higher levels of LHC-II mRNA than corresponding seedlings germinated by red light (14). Other experiments with the plant growth regulator benzyladenine have shown increased synthesis of LHC-II mRNA in dark-grown *Lemna gibba* (9). Enhanced LHC-II mRNA synthesis also resulted following treatment with both red light and benzyladenine compared to only red light treatment (9). Further analyses revealed that benzyladenine may stabilize LHC-II mRNA and thereby increase detectable LHC-II mRNA levels in *Lemna gibba* (10). From our data it can be concluded that in peas GA does not appear to effect LHC-II mRNA levels but may effect the rate of greening by regulating Chl biosynthesis.

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**Table I. Chl a/b Ratios of Pea cvs Progress and Alaska Greened for 4, 24, and 48 h With and Without GA Treatment**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Chl a/b ratios as described by Moran (19)</th>
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<tbody>
<tr>
<td></td>
<td>4 h</td>
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<tr>
<td>Progress</td>
<td>5.8 ± 0.2*</td>
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<tr>
<td>Progress with GA</td>
<td>7.0 ± 0.1</td>
</tr>
<tr>
<td>Alaska</td>
<td>7.0 ± 0.2</td>
</tr>
<tr>
<td>Alaska with GA</td>
<td>7.0 ± 0.3</td>
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*Values are means ± SE for four replicates (10–15 plants per replicate) during each of two (48 h) or three (4 and 24 h) separate greening experiments for a total of either 8 or 12 separate determinations per value.

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**Figure 2.** Accumulation of (A) Chl and (B) LHC-II during light-induced greening of etiolated pea seedlings. (■), cv Progress; (□), cv Alaska; (○), cv Progress with GA added; (●), cv Alaska with GA added. Each point for Chl is the average of at least four replicates (10–15 plants per replicate) from two (2 and 6 h) or three (0, 4, and 24 h) separate greening experiments. For LHC-II each point represents the average of two separate greening experiments, in which two protein extracts were prepared and run in duplicate on two separate single radial immunodiffusion gels. Each value is therefore the average of 16 separate numbers. The error bars lie within the markers for each point for both Chl and LHC-II content.
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LITERATURE CITED