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Regulation of Light-Harvesting Chlorophyll Protein Biosynthesis in Greening Seedlings[1]

A SPECIES COMPARISON

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ABSTRACT

The biosynthesis of the chlorophyll *a/b* binding protein associated with photosystem II (LHC-II) was characterized during light-induced greening of etiolated barley (*Hordeum vulgare* [L.] cv Boone), maize (*Zea mays* [L.] Pioneer 3148), pea (*Pisum sativum* [L.] cv Progress 9), and soybean (*Glycine max* [L.] Merr. cv Ransom 2). Northern blot analysis revealed that pea LHC-II mRNA was present in dark-grown seedlings and accumulated rapidly within 1 hour following illumination with white light. In contrast, the accumulation of LHC-II mRNA was delayed in barley and soybean until 2 to 4 hours after illumination began. Single radial immunodiffusion analysis revealed that LHC-II polypeptides began to accumulate in all species between 4 and 8 hours although the protein was present in detectable levels at earlier times in certain species. In a pattern similar to the LHC-II protein accumulation, chlorophyll accumulated at increased rates between 4 and 8 hours of greening in all species following an initial delay. The absence of coordination between LHC-II mRNA and LHC-II protein accumulation that was clearly observed in pea suggested that transcription is not the factor that limits LHC-II complex formation during chloroplast development. The accumulation of chlorophyll and LHC-II protein appeared to coincide suggesting that chlorophyll biosynthesis may be a factor that limits LHC-II complex formation.

LHC-II[3] is the major light-harvesting Chl-protein complex present in the thylakoid membranes of chloroplasts (31). LHC-II polypeptides are encoded by nuclear genes (7, 10) that are organized into gene families (12, 27). Differences in the organization of individual genes have been detected. For example, *Lemna gibba* contains LHC-II genes with and without introns (15). Substantial evidence indicates that LHC-II polypeptides are synthesized on cytoplasmic ribosomes as precursors and transported into chloroplasts for processing and assembly (9, 14). Regulation of LHC-II genes is known to be controlled by light (3, 13, 30).

Recently, we have shown that stable LHC-II Chl-protein complexes accumulate at different rates in various species during light-induced chloroplast development (5). Relative to maize and soybean, the accumulation of LHC-II complexes was delayed in barley, pea, and wheat. The differences in LHC-II complex accumulation could be caused by species differences in the timing of transcription, translation, assembly of polypeptides and Chl into complexes, or the relative stability of LHC-II Chl-protein complexes during mild SDS extraction. Species differences in the timing of LHC-II mRNA accumulation are known. Dark-grown barley seedlings required 2.5 h to accumulate detectable levels of LHC-II mRNA following a pulse of red light (13). In contrast, low levels of LHC-II mRNA were detected in dark-grown pea seedlings before illumination with white light (11, 30). A critical step in the determination of how transcription or other factors may cause species differences in LHC-II accumulation is that chloroplast development must be compared under the same environmental conditions. Environmental factors can have a major effect on the rate of greening (24; JN Mathis, KO Burkey, unpublished data). In the work presented here, seedlings were germinated and greened under the same light and temperature conditions to minimize environmental differences. The accumulation of LHC-II mRNA, LHC-II polypeptides, LHC-II complexes, and Chl was measured during greening in an attempt to identify steps that limit the overall biosynthetic process.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Seeds were germinated in moist vermiculite at 25°C in the dark. Barley (*Hordeum vulgare* [L.] cv Boone), maize (*Zea mays* [L.] Pioneer 3148), and pea (*Pisum sativum* [L.] cv Progress 9) required a 7 d germination period whereas soybean (*Glycine max* [L.] Merr. cv Ransom 2) was germinated for 5 d. 'Dark' manipulations were performed in the presence of a green safelight constructed from a combination of 2.75 W tungsten illuminator and green filter (Bausch and Lomb 31-35-61).[4] The etiolated seedlings were transferred

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[3] Abbreviations: LHC-II, the major light-harvesting Chl-protein complex associated with photosystem II; MOPS buffer, 20 mM morpholinosulfonic acid, 5 mM sodium acetate, 1 mM EDTA (pH 7.0); DEP, diethylpyrocarbonate; 1 × SSC, 0.15 M sodium chloride and 0.015 M sodium citrate.

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to continuous light at 25 to 27°C. The illumination was provided by a combination of fluorescent and incandescent lamps that produced an intensity of 200 to 250 $\mu E \cdot m^{-2} \cdot s^{-1}$ at the top of the seedlings.

Preparation of cDNA probes. Probes for barley (13) and pea (3) LHC-II mRNA were the gifts of K. Apel (University of Kiel, Kiel, Federal Republic of Germany) and N.-H. Chua (Rockefeller University, New York, NY). Plasmid DNAs for each of these probes were prepared by the method of Norgard (26). The probe for pea and soybean LHC-II mRNA was obtained by digesting the plasmid pAB96 with *PstI*, followed by electroelution (33) of an 800 nucleotide fragment from a 5% polyacrylamide gel (22). The intact plasmid pHvLF2 was used as probe DNA specific for barley LHC-II mRNA. Radioactive cDNA probes were prepared using a BRL nick translation kit.

Preparation of Total RNA. Total RNA was isolated at harvest time from both dark-grown and illuminated seedlings. Barley, maize, and pea leaves and soybean cotyledons were ground in liquid nitrogen in a mortar which had been baked at 220°C for 4 h and then cooled. A portion of the frozen tissue powder was suspended in 20 mM sodium acetate, 1% SDS (pH 5.2) at a ratio of 100 ml per g of frozen tissue, and extracted three times with hot phenol (65°C) as described by Scherrer (28). The remainder of the frozen tissue powder was stored at −80°C. Purity and concentration of total cellular RNA were determined spectrophotometrically (22). All glassware used in RNA extraction were baked and solutions treated with DEP (22) to remove contaminating ribonucleases.

Northern Blot Analysis. Northern blot analyses were performed using modifications of the methods outlined by New England Nuclear for their Gene Screen Transfer Membrane. Total RNA was denatured at 65°C in 1 × MOPS buffer containing 50% formamide and 6% formaldehyde. RNA samples (10 μg) were separated in 1.8% agarose gels containing 6% formaldehyde (20). Electrophoresis was performed for 18 to 20 h at 20 V in 1 × MOPS buffer with buffer recirculation. Gels were treated with 50 mM NaOH and 10 mM NaCl, neutralized in 200 mM sodium acetate, and the RNA transferred to Gene Screen as recommended by New England Nuclear.

Prehybridization of membrane filters was performed in a solution containing 50% formamide, 0.04% polyvinyl-pyrrolidone (mol wt 40,000), 0.04% BSA, 0.04% ficoll (mol wt 400,000), 5 × SSC, 1% SDS, and 100 μg/ml salmon sperm DNA. Prehybridization was performed in Seal-A-Meal bags 16 to 20 h at 37°C with constant agitation (150 rpm). Denatured cDNA probes (boiled 3 min in 200 μl of prehybridization solution) were then added directly to the solution used for prehybridization. Hybridization was then performed 40 to 46 h at 37°C with constant agitation. Following hybridization, membrane filters were washed with either 1 × SSC, 0.1% SDS or 0.1 × SSC, 0.1% SDS to vary stringency (22). Filters were then wrapped in plastic wrap and autoradiography was conducted at −70°C using Kodak XAR5 film in Kodak X-Omatic cassettes with intensifying screens.

Preparation of Antibodies against LHC-II Polypeptides. LHC-II polypeptides were purified by a combination of two procedures. Initially, LHC-II protein was purified from thylakoid membranes of 2 to 3 week old greenhouse grown barley by detergent extraction and salt precipitation (18). The precipitated LHC-II protein was solubilized in 2 mM Tris-maleate (pH 7.0), 10% glycerol, and 0.88% octyl-β-D-glucopyranoside (detergent/Chl = 20) for 30 min at room temperature (6). SDS was added to a final concentration of 0.22% and the proteins were separated in preparative 10% acrylamide gels (17) at 4°C. The 'green' oligomeric LHC-II protein (CP64 in reference 6) was electroeluted at 4°C in the presence of 2.2 mM glycine, 0.3 mM Tris, and 0.05% lithium dodecyl sulfate. The LHC-II protein was concentrated and then incubated in 0.3 M Tris-HCl (pH 8.8), 10% glycerol, and 1% SDS (SDS/Chl = 20–40) for 30 min at room temperature to produce the monomeric form of the LHC-II protein. The monomeric LHC-II protein was separated on a second preparative gel as described above. The purified LHC-II polypeptides were electroeluted, dialyzed overnight at room temperature against PSS buffer (50 mM NaH_2PO_4 [pH 7.0], 0.15 M NaCl, 1% SDS), and precipitated with cold acetone. The precipitated LHC-II protein was redissolved in PSS buffer at a protein concentration of 0.5 to 1.0 mg/ml and used to immunize rabbits following a standard procedure (8).

Polyacrylamide gel electrophoresis (19) was performed on soluble and thylakoid membrane protein fractions from 2 week old barley and pea seedlings grown in the greenhouse. Proteins were transferred to Gene Screen, blocked and incubated in a 1 to 25,000 dilution of LHC-II antisera by the methods of the vendor, New England Nuclear. LHC-II polypeptides were detected on Western Blots with horseradish peroxidase conjugated goat anti-rabbit antibodies by the methods of the vendor, Biorad.

Single Radial Immunodiffusion Analysis. The LHC-II protein content of whole tissue extracts was determined by single radial immunodiffusion (32). Gel plates (15 × 15 × 0.3 cm) consisted of 1% agarose in buffer (pH 7.0) that contained 50 mM Tris-HCl, 0.1 mM EDTA, 5 mM $MgSO_4$, 0.9% NaCl, 15 mM NaN_3, 1% Triton X-100, and rabbit antiserum (37.5 μl per ml of gel) specific for LHC-II polypeptides. Protein extracts (prepared as described below) were loaded into 2.5 mm diameter wells by repetitive addition of 10 μl aliquots with a 10 min period between each addition to allow for uptake of the sample into the gel. Barley LHC-II protein standards were prepared by serial dilution of the purified LHC-II protein in PSS buffer with 4% Triton X-100. Known quantities of barley LHC-II protein (0.025–3.0 μg) were added to gel wells as described for the unknowns. The gel was incubated in sealed containers for 48 h at room temperature to allow radial diffusion and complete precipitation of LHC-II protein. The gel was washed extensively with 0.9% NaCl and then water to remove nonprecipitated protein. The precipitin rings were stained with Coomassie blue. The gel was dried and the area of the precipitin rings measured by planimetry after enlargement.

Whole tissue protein extracts were prepared from aliquots of the same frozen tissue powder used to isolate total RNA. Frozen tissue powder that had been stored at −80°C was homogenized in 50 mM NaH_2PO_4 (pH 7.0), 2 mM phenylmethylsulfonyl fluoride in a ratio of 0.1 g of tissue per ml of buffer. SDS was then added to the extract to a final concentration of 2%. The extract was heated at 50°C for 15 min and the insoluble material removed by centrifugation. No additional protein could be released by a second extraction of the insoluble material. The extracts were stored at −80°C. Before analysis of the LHC-II content, the extracts were thawed and 20% Triton X-100 added to a final concentration of 4%.

Chl and Protein Determination. Chl was extracted from tissue samples with dimethylformamide and the Chl content measured as described by Moran (25). The protein concentration was measured by the Lowry method (21) with ovalbumin as a standard.

Analysis of LHC-II Complex Accumulation. Plastid membranes were isolated from illuminated barley, maize, and pea leaves or soybean cotyledons as previously described (5). Membranes were washed and the Chl-protein composition analyzed by mild SDS-PAGE as previously reported (4).

RESULTS

Accumulation of LHC-II mRNA during Light-Induced Greening. In pea, LHC-II mRNA was present at low levels prior to exposure to white light (Fig. 1A). The presence of LHC-II mRNA

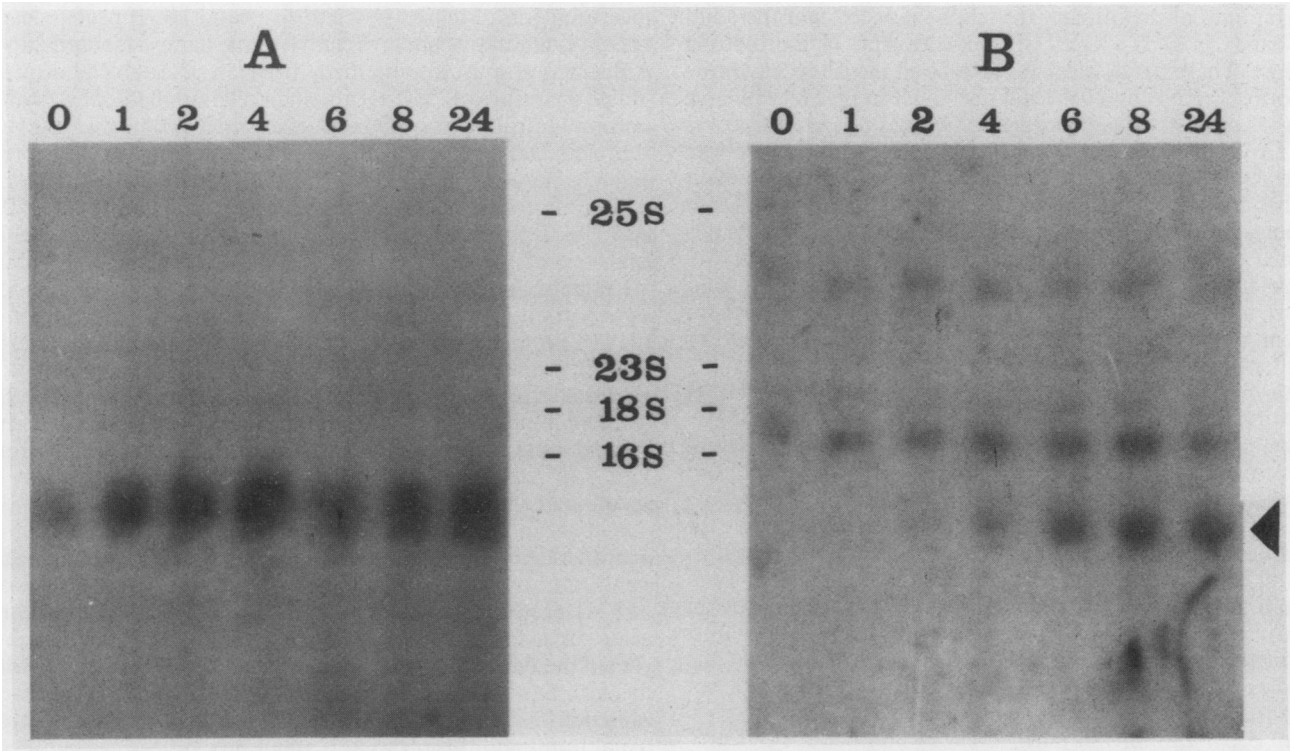

FIG. 1. Northern blot analysis of 10 μg samples of total RNA isolated from etiolated seedlings that had been greened under continous white light for 0, 1, 2, 4, 6, 8, and 24 h. Pea LHC-II specific cDNA was used as a probe. A, Pea RNA, 12 h exposure of autoradiogram; B, soybean RNA, 9 d exposure of autoradiogram. (◀), Soybean LHC-II mRNA.

in pea before white light illumination may have been induced by the green safelight because pea is known to have a low fluence response (16). However, low levels of LHC-II mRNA have been observed in dark grown pea seedlings by others (11, 30). Levels of pea LHC-II mRNA increased relative to total RNA during the first 2 h of greening and were relatively constant throughout the 24 h greening period.

Probing of soybean RNA with pea LHC-II cDNA revealed three bands of homology (Fig. 1B). Based on mol wt (3, 13), the band with greater relative mobility than 16S rRNA was identified as LHC-II mRNA. Soybean LHC-II mRNA was observed as early as 2 h after the greening process had begun. Soybean LHC-II mRNA then increased relative to total RNA until 6 h and remained constant at later times. Two higher mol wt bands were present from 0 to 24 h in soybean. These bands were observed because extended x-ray film exposure times were required to reveal the soybean LHC-II mRNA developmental profile under heterologous probing conditions. Extended exposure of pea blots revealed additional bands of similar mol wt as those observed in soybean (data not shown).

In the case of barley RNA (Fig. 2), LHC-II mRNA was present in very low levels as early as 2 h (requiring extended exposure of the x-ray film to see clearly). A dramatic increase in barley LHC-II mRNA, relative to total RNA, occurred between 2 and 4 h after exposure to light. After 6 h, the level of LHC-II mRNA remained constant. Others have observed a delay in the accumulation of LHC-II mRNA in etiolated barley after a flash of red light (13). In our experiments, illumination with continuous white light produced the same type of response. Two additional bands of homology were also observed in barley RNA when blots were exposed to x-ray film for extended periods of time. One of the additional bands observed in barley RNA had a mol wt similar to the additional band of lower mol wt observed in soybean and pea. The second additional band in barley RNA had an intermediate mol wt compared with the two additional bands observed in soybean and pea. Thus, the additional RNA species detected with an LHC-II cDNA probe in barley formed a different banding pattern than did the additional RNA species in soybean and pea.

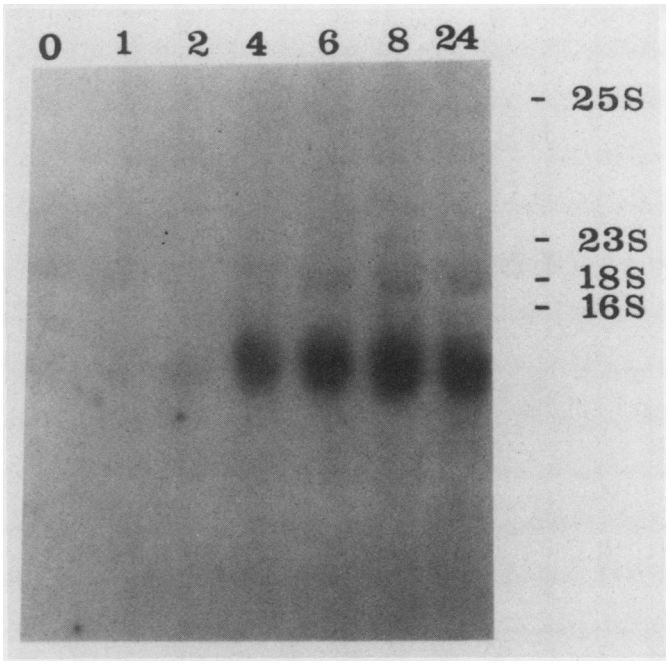

FIG. 2. Northern blot analysis of 10 μg samples of total RNA isolated from etiolated barley seedlings that had been greened under continuous white light for 0, 1, 2, 4, 6, 8, and 24 h. Barley LHC-II specific cDNA was used as a probe. The autoradiogram was exposed for 40 h.

Several lines of preliminary evidence suggested that the additional bands in each species were not artifacts of the blotting procedure. These bands did not correspond in size to any prevalent form of rRNA and were not decreased in intensity relative to LHC-II mRNA by high stringency washes (0.1 × SSC, 0.1% SDS). This observation indicated that the additional bands were not the result of nonspecific binding of probe DNA. The additional bands were bound to poly(U)-sepharose and thus appeared to be polyadenylated. This would indicate these additional bands were mRNAs of nuclear origin.

Maize LHC-II mRNA was observed as early as 2 h after exposure to light (data not shown). However, Northern blots of sufficient quality to show conclusive developmental patterns were not obtained because homology differences between either the pea or barley LHC-II cDNA probes and maize LHC-II mRNA made heterologous probing difficult.

Accumulation of LHC-II Protein during Light-Induced Greening. Specific rabbit antibodies raised against LHC-II protein from barley were used to measure LHC-II accumulation in greening barley, maize, pea, and soybean. The antibodies were specific for LHC-II apoproteins as determined by Western blotting against barley thylakoid membrane preparations (Fig. 3) as well as pea thylakoid membranes (data not shown). The absence of an antibody reaction on Western blots of barley and pea soluble leaf proteins served as an additional control to show that the antibodies were specific for LHC-II polypeptides (data not shown).

When equal amounts of purified barley and pea LHC-II protein were assayed by single radial immunodiffusion, the area of the precipitin ring was less for pea than barley. This indicated that the cross-reactivity of the barley LHC-II antibodies was different for each of the species. For the work presented here, purified barley LHC-II was used as a standard (Fig. 4). Therefore, measurements of maize, pea, and soybean LHC-II protein levels are not quantitative but are relative to the barley standard curve. A distinction must also be made between detectable and measurable quantities of LHC-II protein in relation to the single radial immunodiffusion assay. Quantities as small as 0.025 μg of LHC-II protein could be detected in an aliquot of whole tissue protein extract. However, the limitations associated with the determination of precipitin ring areas required that a minimum of 0.1 μg LHC-II protein be present in an aliquot before the level of LHC-II protein could be measured reliably by planimetry. Thus, 'detectable' and 'measurable' have distinct meanings in the following discussions because the assay can detect the presence of LHC-II protein in samples where the actual amount cannot be measured accurately.

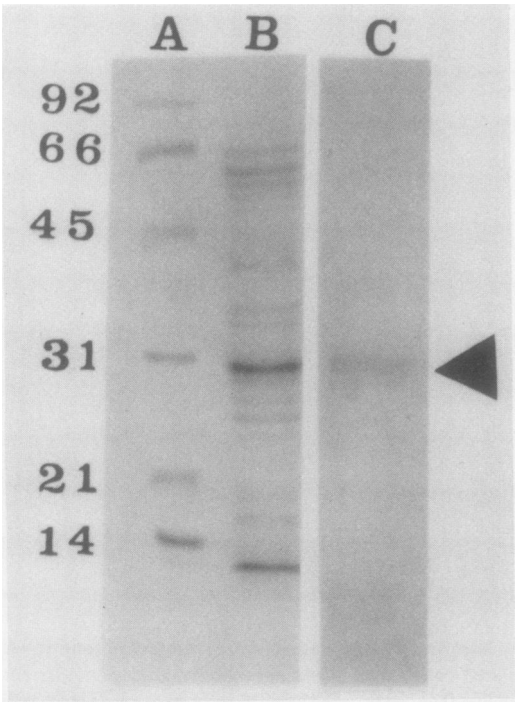

FIG. 3. Western blot analysis of barley thylakoid membrane proteins heated to 50°C and loaded on a 10% polyacrylamide gel. A, Biorad low mol wt standards with polypeptide sizes given in kilodaltons; B, 25 μg protein sample of barley LHC-II thylakoid membranes; and C, an identical lane from the same gel as lane (B) was transferred to gene screen and LHC-II polypeptides detected with LHC-II antibodies. (◀), LHC-II polypeptides.

Measurable levels of pea LHC-II protein were present 1 h after the beginning of light-induced greening in pea (Fig. 4). These levels did not increase significantly during the time period from 1 to 4 h. After 4 h of greening, pea LHC-II protein began to accumulate in larger quantities (Figs. 4 and 5).

A soybean LHC-II precipitin ring was observed after 4 h of light-induced greening (Fig. 4). These levels did not increase significantly until after 6 h of greening (Fig. 5). After 6 h, soybean LHC-II protein began to accumulate in larger quantities. Soybean cotyledon extracts presented a unique problem that prevented the detection of soybean LHC-II protein before 4 h with this technique. A component of the 0 to 2 h cotyledon extracts caused diffuse background Coomassie staining around the single radial immunodiffusion well. If small precipitin rings had been present, they would not have been observed. However, green gels showed that stable LHC-II membrane complexes were present in soybean as early as 2 h indicating the presence of LHC-II protein (Fig. 7).

Detectable quantities of maize LHC-II protein were found after 1 h of light-induced greening (Fig. 4). Measurable quantities of maize LHC-II protein were present in the tissue beginning at 2 h. Although a low level of LHC-II protein was present in maize leaf tissue from 1 to 4 h (Fig. 5), LHC-II protein did not begin to accumulate in larger quantities until 6 h.

Barley LHC-II protein was detected after 2 h of light-induced greening, but measurable levels were not present until 4 h (Fig. 4). After 4 h, barley LHC-II protein began to accumulate in larger quantities (Figs. 4 and 5).

Accumulation of Chl during Light-Induced Greening. The accumulation of Chl followed the same general pattern in barley, maize, pea, and soybean although the absolute amount of Chl present in the tissue was species dependent (Fig. 6). A lag in Chl accumulation was observed during the first 2 to 4 h of greening followed by an increased rate of Chl accumulation at later times. This pattern is commonly observed in experiments where etiolated seedlings are greened in continuous light (4, 29).

Accumulation of LHC-II Chl-Protein Complexes during Light-Induced Greening. Differences were observed in the accumulation of LHC-II complexes (Fig. 7). The amount of Chl present in plastid membranes after 1 h of greening was extremely small so that no Chl-protein complexes or free pigment could be detected in tube gels after mild SDS-PAGE analysis. Stable LHC-II complexes in soybean and maize plastid membranes represented a large percentage of the total Chl after 2 h of greening. The level of soybean and maize LHC-II complexes increased as chloroplast development proceeded. In pea, stable LHC-II complexes were detected after 3 h of greening, but did not represent a large percentage (>10%) of the total Chl until 4 h. In barley, accumulation of stable LHC-II complexes was delayed for the longest period of time relative to the other species examined. No LHC-II complexes were detected in barley plastid membranes after 4 h of greening. LHC-II complexes accumulated rapidly in barley between 4 and 6 h. The results clearly demonstrate species

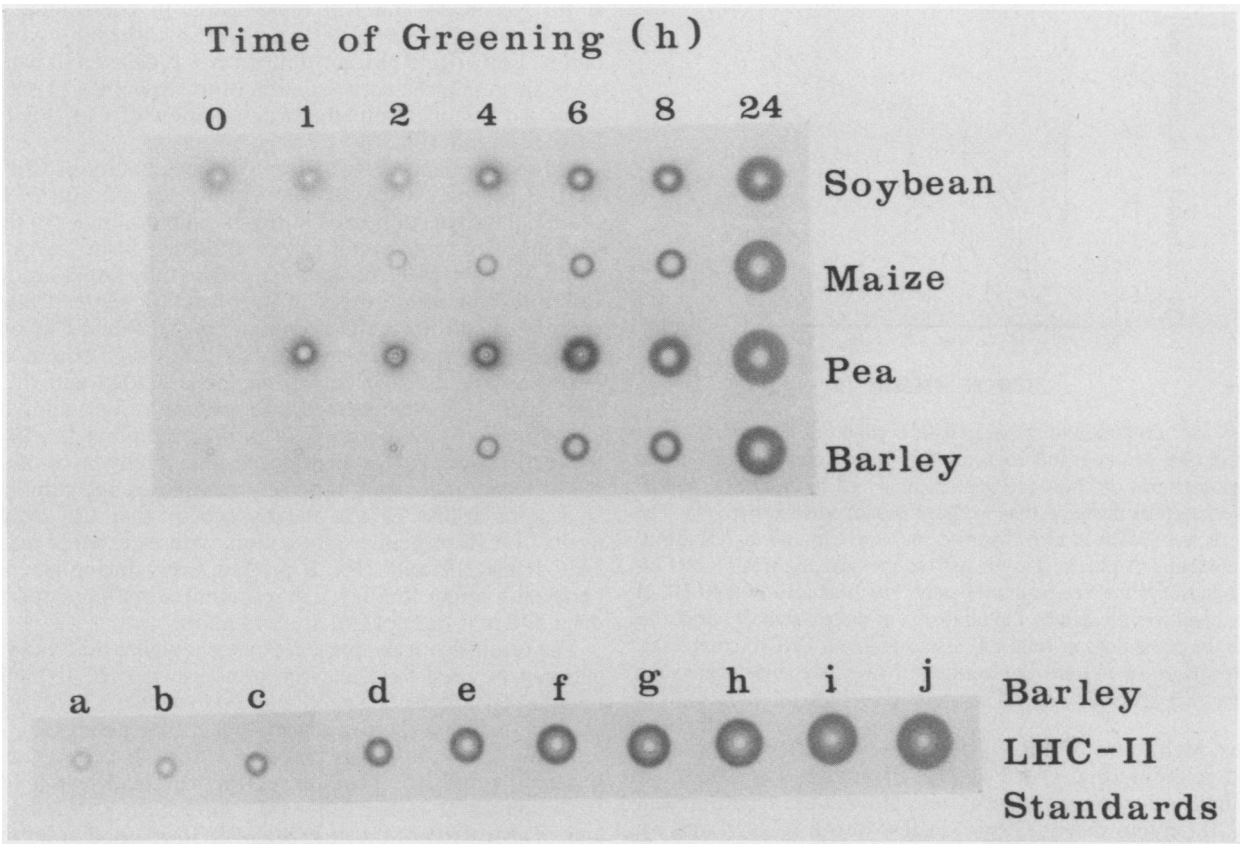

FIG. 4. Single radial immunodiffusion analysis of LHC-II protein in greening seedlings. Thirty μl aliquots of whole tissue extracts were prepared and analyzed as described in "Materials and Methods." A series of barley LHC-II standards containing 0.025 (a), 0.05 (b), 0.1 (c), 0.25 (d), 0.5 (e), (0.75 (f), 1.0 (g), 1.5 (h), 2.0 (i), and 3.0 (j) μg of purified protein were analyzed simultaneously for comparison with the samples from greening tissue.

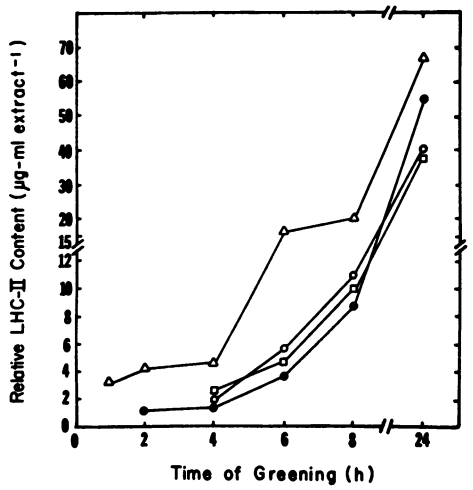

FIG. 5. Accumulation of LHC-II protein in greening seedlings. LHC-II protein content relative to barley LHC-II standards was calculated from the average of two replicates of data similar to that presented in Figure 4; barley (○), maize (●), pea (△), and soybean (□).

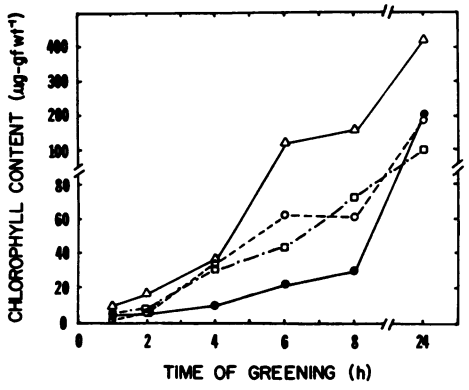

FIG. 6. Accumulation of Chl in seedlings during light induced greening; barley (○), maize (●), pea (△), and soybean (□).

differences in the time course for accumulation of stable LHC-II complexes during chloroplast development. However, during early stages of chloroplast development, differences between species in the stability of LHC-II complexes appear to be the reason for differences in LHC-II complex accumulation (Fig. 7; see "Discussion").

DISCUSSION

The present study was initiated to determine how transcriptional and posttranscriptional factors may control the accumulation of LHC-II Chl-protein complexes in the thylakoid membranes of higher plants. The accumulation of LHC-II mRNA, LHC-II protein, and Chl during light-induced greening of etiolated seedlings was compared in several different plant species. To minimize environmental factors that are known to affect the rate of greening in a single species (24; JN Mathis, KO Burkey, unpublished data), seedlings were carefully germinated and greened under the same conditions so that species differences could be observed.

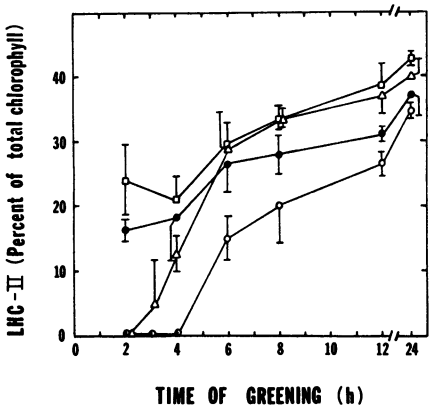

FIG. 7. Accumulation of stable LHC-II complexes in greening barley (○), maize (●), pea (△), and soybean (□). Plastid membranes isolated after various times of illumination were analyzed by mild SDS-PAGE using the identical protocol that we have described elsewhere (5). The 'green' gels were scanned at 675 nm to measure Chl and at 720 nm to obtain a baseline. A planimeter was used to measure the area of the Chl-protein bands and the free pigment bands. The peak area of the LHC-II complex band was divided by the total area of the gel scan of interest to calculate the percentage of total Chl associated with LHC-II complexes. The percentages (± SE) are the average of two independent greening studies for each species.

In pea, high levels of mRNA were observed after 1 h of light-induced greening (Fig. 1A). Pea LHC-II protein was detected 1 h after light-induced greening was initiated, but the accumulation of LHC-II protein did not begin until 4 to 6 h (Figs. 4 and 5). The pea results indicate that factors other than mRNA accumulation limit LHC-II protein accumulation. This lack of coordination between LHC-II mRNA and protein accumulation has been observed by others (23) in experiments where the levels of LHC-II mRNA and protein were measured at different positions along an expanding maize leaf. These results suggest that some factor other than transcription may limit the biosynthesis of LHC-II Chl-protein complexes.

The accumulation of both LHC-II mRNA and LHC-II protein appeared to be more closely coordinated in barley and soybean than was the case for pea. In barley (Fig. 2) and soybean (Fig. 1B), LHC-II mRNA is detected after 2 h of light-induced greening. LHC-II protein is detected by single radial immunodiffusion as early as 2 h in barley (Fig. 4). In soybean, LHC-II protein could not be detected until 4 h by single radial immunodiffusion because of problems associated with cotyledon extracts. However, mature LHC-II Chl-protein complexes were detected in soybean at 2 h after the start of greening, indicating the presence of LHC-II protein at this time (Fig. 7). In both barley and soybean, LHC-II mRNA relative to total RNA increased to maximum levels between 2 and 6 h of light-induced greening. LHC-II protein began to accumulate in barley and soybean between 4 and 8 h (Fig. 5).

In the four species examined, the accumulation of Chl and LHC-II protein followed a similar pattern. The accumulation of LHC-II protein began at about 4 h after the onset of light-induced greening (Fig. 5), although protein was present in small quantities at earlier times in certain species (Figs. 4 and 7). A delay in Chl accumulation was observed in each species, followed by an increase in the rate of Chl accumulation that began after 4 h of greening (Fig. 6). One interpretation of this result is that Chl biosynthesis may be the factor that limits LHC-II protein accumulation. The accumulation of mRNA is clearly not the limiting factor in pea because high levels of LHC-II mRNA (Fig. 1A) were present several hours before LHC-II protein accumulated in large quantities (Fig. 5). The relative importance of LHC-II mRNA levels and Chl biosynthesis in controlling LHC-II protein accumulation could not be distinguished in soybean and barley. LHC-II mRNA accumulation was delayed in barley and soybean so that the accumulation of the mRNA was not clearly separated in time from the accumulation of Chl (Fig. 6) and LHC-II protein (Fig. 5).

Indirect evidence suggests that the availability of Chl during LHC-II complex assembly may control the amount of LHC-II protein that is accumulated in thylakoid membranes in the form of stable Chl-protein complexes. Etiolated bean leaves illuminated with intermittent light synthesize only small amounts of Chl and incorporate this Chl into reaction center Chl-protein complexes and not LHC-II complexes (2). When Chl synthesis is limited by transfer of greening seedlings from continous light into the dark, LHC-II complexes are degraded and the Chl *a* from LHC-II is incorporated into reaction center complexes (1, 29). Therefore, the presence of limited amounts of Chl during the early stages of chloroplast development may favor the assembly of reaction center Chl-protein complexes and inhibit LHC-II accumulation. To test the hypothesis that Chl availability limits LHC-II protein accumulation, we are currently measuring LHC-II mRNA and LHC-II protein levels during greening experiments where the light environment is manipulated to produce different rates of Chl accumulation.

The results from the present study show that mild SDS 'green' gels can be used to detect the presence of LHC-II membrane complexes but are not a good method to measure LHC-II protein content, particularly in greening tissue. In general, LHC-II protein (Figs. 4 and 5) was detected 1 to 2 h before the initial observation of LHC-II complexes (Fig. 7). The detection of LHC-II complexes at earlier times may have been prevented by the lack of sensitivity of the mild SDS-PAGE method or because the LHC-II complexes were not stable during the required detergent extraction. High levels of free Chl have been detected during mild SDS-PAGE analysis of thylakoids from greening seedlings which indicated that the Chl-protein complexes are unstable during the initial stages of chloroplast development (5). Results from the present study suggested that the initial LHC-II complexes may be more stable in certain species. For example, maize contained higher levels of stable LHC-II complexes during the first 4 h of greening than did pea (Fig. 7), but pea contained higher relative amounts of LHC-II protein (Fig. 5). Therefore, the level of LHC-II complexes that could be detected by mild SDS-PAGE during the first 4 h of greening did not reflect the level of LHC-II protein. Between 4 and 8 h of greening, both LHC-II protein and LHC-II complexes increased relative to the 4 h level. This observation suggested that changes in the level of LHC-II complexes more accurately reflects changes in LHC-II protein during later stages of chloroplast development.

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