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Communication

Novel Light-Regulated Chloroplast Thylakoid Membrane Protein

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ABSTRACT

A 64 kilodalton chloroplast membrane polypeptide was dependent on growth irradiance with 10-fold greater quantities of the protein present in barley (*Hordeum vulgare*) grown under 500 micromoles of photons per square meter per second compared with growth at 50 micromoles per square meter per second. The concentration of the protein was sensitive to changes in irradiance, with a slow time course for the response (days) similar to other reported light acclimation processes. The polypeptide also was observed in maize (*Zea mays*), oats (*Avena sativa*), and wheat (*Triticum aestivum*), but not in soybean (*Glycine max* Merr). The 64 kilodalton polypeptide did not correspond to any thylakoid membrane protein with an assigned function, so its structural or regulatory role is not known.

Light serves as both an energy source and a regulatory signal for photosynthesis. The irradiance and spectral quality of light surrounding an individual leaf controls the capacity of that leaf to conduct photosynthesis (1–3, 10) and associated processes such as chloroplast electron transport (3, 6, 9, 10) and photophosphorylation (4, 7, 10). Light regulates these photosynthetic activities by controlling the levels of specific chloroplast proteins associated with each function. The concentrations of several proteins and protein-complexes are known to be light-regulated including ribulose-1,5-bisphosphate carboxylase/oxygenase (3, 16), the light-harvesting Chl-protein complexes (8, 14), the PSII reaction center (2, 4, 5, 9, 10, 13, 17), the Cyt *b/f* complex (1, 4, 5, 9, 10, 13, 17), and chloroplast ATP synthetase (1, 4, 5, 7). Typically, a 5- to 10-fold difference in irradiance causes the concentration of these protein components to vary by a factor of two or less. In this study, a new light-regulated thylakoid membrane polypeptide was identified that varied by a factor of 10 in response to irradiance and was correlated with light acclimation processes.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Plants were grown in 1-L pots of soil in controlled environment chambers with a 16-h photoperiod. Maximum irradiance within the chamber was 500 μmol photons m^{-2} s^{-1}. Cheesecloth neutral density filters were used to produce a low irradiance of 50 μmol photons m^{-2} s^{-1} in a section of the chamber (8). Barley (*Hordeum vulgare* cv WB158-1), oats (*Avena sativa* cv Brooks), and wheat (*Triticum aestivum* cv Pioneer 2548) were grown at 21°C. Soybean (*Glycine max* Merr. cv Young) and maize (*Zea mays* cv Pioneer 3184) were grown at 25°C. After emergence, seedlings were thinned to minimize self-shading. Plants were grown for 2 weeks under high or low irradiance conditions prior to analysis. For light acclimation experiments, 8 d old barley plants were transferred between high and low irradiance environments and the acclimation response examined for a period of 1 week.

Thylakoid Membrane Isolation

Primary leaves were harvested at the beginning of the photoperiod. Thylakoid membranes were isolated from leaf tissue as described previously (8). The grind buffer contained 0.4 M sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 0.2% (w/v) BSA, and 50 mM Tricine-NaOH (pH 7.8). The membranes were washed once in cold grind buffer, resuspended in grind buffer, and stored on ice during electron transport measurements. Membranes were then frozen in liquid nitrogen before long term storage at −80°C.

Chl Determination

Chl content of fresh leaf discs and thylakoid membrane preparations was determined by extraction of pigments with dimethylformamide followed by spectrophotometric assay (15).

Analysis of Thylakoid Membrane Activity and Polypeptide Composition

Uncoupled whole chain photosynthetic electron transport was assayed as 2,6-dichlorophenolindophenol reduction with water as the electron donor (11). Details have been published elsewhere (9).

PAGE was conducted in 10% (w/v) gels using the procedure of Laemmli (12) with lithium dodecylsulfate substituted for SDS to facilitate analysis at low temperature. Thylakoid membranes were washed twice in 10 mM Na-PPi (pH 7.5) and resuspended in the same buffer prior to electrophoresis. This washing procedure completely removed BSA and any trace of soluble proteins present after the isolation procedure. Washed membranes were solubilized on ice for 15 min at a

detergent/Chl ratio (w/v) of 40. Gels were loaded on a Chl basis (15 μg/lane) and electrophoresis was conducted at 8°C. Gels were stained with Coomassie blue and destained prior to analysis with an LKB[1] laser densitometer. Relative concentrations of the 64 kD polypeptide were determined by integration of gel scan peak areas.

RESULTS AND DISCUSSION

PAGE analysis of chloroplast thylakoid membranes revealed an irradiance-dependent polypeptide with an apparent molecular mass of 64 kD. In barley, oats, and wheat, the polypeptide migrated as a clearly resolved band between the apoprotein of the PSI reaction center and the α-subunit of CF_1[2] (Fig. 1). The polypeptide was observed before and after purification of thylakoid membranes on sucrose step gradients using the procedure of Burkey and Wells (1) (data not shown). Membranes from plants grown under high irradiance contained greater quantities of the protein than membranes from low irradiance plants. To provide a point of reference, the staining intensity of the 64 kD polypeptide was 10 to 15% of the α-subunit of CF_1 under high irradiance conditions where maximum levels of the 64 kD polypeptide were observed. The results were the same if membranes were solubilized on ice (Fig. 1) or at 50°C (data not shown) prior to electrophoresis. Ice solubilization was preferred because a majority of the PSI reaction center migrated as a native complex at 120 kD so that quantification of the 64 kD polypeptide by densitometry was more convenient. In maize, a polypeptide of slightly larger molecular mass (~64 kD for maize compared with approximately 63 kD for barley, oats, and wheat) and similar irradiance response was observed in high concentration gradient gels (data not shown) but was not resolved from the PSI apoprotein in the 10% gels presented here. No analogous polypeptide was observed in soybean thylakoid membranes under any electrophoresis condition tested, although this does not eliminate the possible co-migration with another polypeptide. Until a more specific assay for the polypeptide can be developed, the presence of such a protein in soybean membranes cannot be ruled out.

Light acclimation experiments were conducted to quantify the irradiance dependence of the 64 kD polypeptide and evaluate the time course of the response. Barley was selected for this work because the polypeptide was clearly resolved in the gels and light acclimation has been studied in this species (8, 9). Plants grown under an irradiance of 500 μmol photons m^{-2} s^{-1} contained approximately 10-fold more of the 64 kD polypeptide than plants grown under 50 μmol photons m^{-2} s^{-1} (compare open and closed circles in Fig. 2A). This effect was much larger than the irradiance effects reported for other chloroplast components. In studies that utilized similar illumination treatments, ribulose-1,5-bisphosphate carboxylase/oxygenase (16), the number of PSII reaction centers (4, 5, 9, 10, 13), Cyt *f* (4, 5, 9, 10, 13), and the ATP synthetase (4, 5, 7) varied by a factor of two or less.

The concentration of the 64 kD polypeptide was sensitive to changes in irradiance. When plants were transferred between high and low irradiance conditions, the relative amount of polypeptide associated with the membrane changed to reflect the controls in new light environment (Fig. 2A). The time course of the response was similar to changes in Chl *a*/*b* ratio (Fig. 2B) and photosynthetic electron transport (Fig.

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture or the North Carolina Agricultural Research Service and does not imply its approval to the exclusion of other products that may also be suitable.

[2] Abbreviation: CF_1, chloroplast coupling factor.

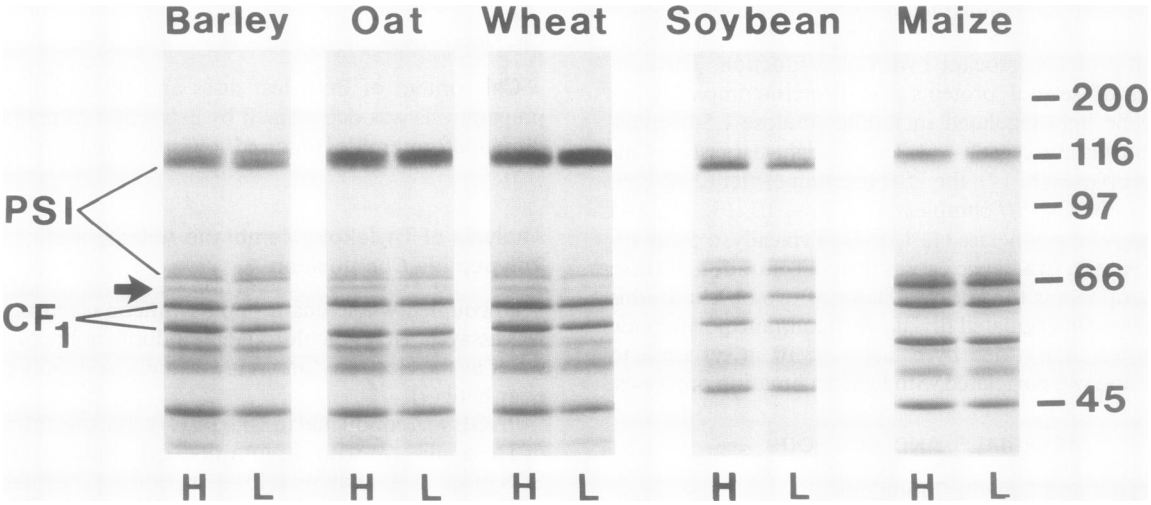

Figure 1. Electrophoretic identification of the 64 kD irradiance-dependent polypeptide. Chloroplast thylakoid membrane polypeptides were separated in 10% gels as described in "Materials and Methods." The high molecular mass region of the gel is presented with the 64 kD polypeptide indicated with a heavy arrow. For reference, both the native complex (upper) and apoprotein (lower) of PSI are indicated as well as the α (upper)- and β (lower)-subunits of CF_1. H and L indicate membranes from plants grown under high or low irradiance, respectively. Molecular masses are indicated in kD.

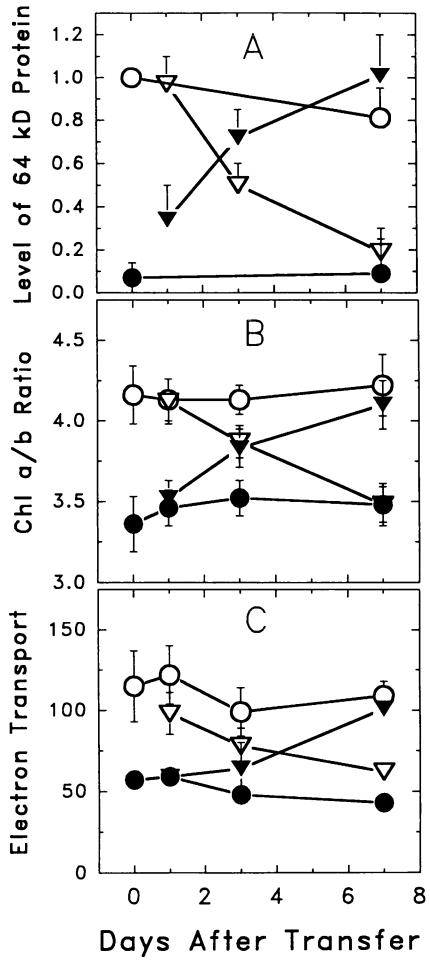

Figure 2. Correlation of the 64 kD polypeptide with light acclimation. Barley seedlings were grown under high (○) or low (●) irradiance conditions for 8 d. Plants were then transferred from high to low (△) or from low to high (▲) and all treatments monitored for an additional 7 days. A, Effect of growth irradiance on the level of 64 kD polypeptide. The concentration of the 64 kD polypeptide was determined from gel scan peak areas. The data were normalized to high irradiance controls at the time light transfer treatments were imposed. B, Effect of growth irradiance on Chl *a*/*b* ratio. Chl *a*/*b* ratio was determined by analysis of pigments in fresh leaf discs. C, Effect of growth irradiance on photosynthetic electron transport capacity measured as 2,6-dichlorophenolindophenol reduction (mmol 2,6-dichlorophenolindophenol mol Chl^{-1} s^{-1}) in isolated thylakoid membranes. See "Materials and Methods" for details. Each point is the average of three independent experiments with error bars representing one SD.

2C), two parameters that are known to be involved in the light acclimation process (3, 6, 8, 9). For each parameter in Figure 2, significant differences were observed within 1 to 3 d after transfer, whereas the complete response required 7 d. The slow acclimation kinetics observed here were typical of light acclimation processes that require the commitment of nutrients and metabolic energy to alter the biochemical composition of the leaf (3, 6, 8, 9, 16).

The 64 kD polypeptide did not correspond to any intrinsic or extrinsic thylakoid membrane protein to which a function has been assigned. Thus, the role of this protein remains unclear. If it is true that the polypeptide is present in certain species (*e.g.* barley) but not others (*e.g.* soybean), then the protein may be a previously unrecognized regulatory component. This interpretation is supported by the much larger irradiance effect on the concentration of the 64 kD protein compared with membrane components that serve required structural or functional roles in the chloroplast thylakoid membrane.

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LITERATURE CITED

1. **Burkey KO, Wells R** (1991) Response of soybean photosynthesis and chloroplast membrane function to canopy development and mutual shading. Plant Physiol **97:** 245–252
2. **Chow WS, A Melis, Anderson JM** (1990) Adjustments of photosystem stoichiometry in chloroplasts improve the quantum efficiency of photosynthesis. Proc Natl Acad Sci USA **87:** 7502–7506
3. **Chow WS, Anderson JM** (1987) Photosynthetic responses of *Pisum sativum* to an increase in irradiance during growth I. Photosynthetic activities. Aust J Plant Physiol **14:** 1–8
4. **Chow WS, Anderson JM** (1987) Photosynthetic responses of *Pisum sativum* to an increase in irradiance during growth II. Thylakoid membrane components. Aust J Plant Physiol **14:** 9–19
5. **Chow WS, Hope AB** (1987) The stoichiometries of supramolecular complexes in thylakoid membranes of spinach chloroplasts. Aust J Plant Physiol **14:** 21–28
6. **Davies EC, Chow WS, LeFay JM, Jordan BR** (1986) Acclimation of tomato leaves to changes in light intensity; effects on the function of the thylakoid membrane. J Exp Bot **37:** 211–220
7. **Davies EC, Jordan BR, Partis MD, Chow WS** (1987) Immunochemical investigation of thylakoid coupling factor protein during photosynthetic acclimation to irradiance. J Exp Bot **38:** 1517–1527
8. **De la Torre WR, Burkey KO** (1990) Acclimation of barley to changes in light intensity: chlorophyll organization. Photosyn Res **24:** 117–125
9. **De la Torre WR, Burkey KO** (1990) Acclimation of barley to changes in light intensity: photosynthetic electron transport activity and components. Photosyn Res **24:** 127–136
10. **Evans JR** (1987) The relationship between electron transport components and photosynthetic capacity in pea leaves grown at different irradiances. Aust J Plant Physiol **14:** 157–170
11. **Izawa S** (1980) Acceptors and donors for chloroplast electron transport. Methods Enzymol **69:** 413–434
12. **Laemmli UK** (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227:** 680–685
13. **Lee W-J, Whitmarsh J** (1989) Photosynthetic apparatus of pea thylakoid membranes: response to growth irradiance. Plant Physiol **89:** 932–940
14. **Leong T-Y, Anderson JM** (1984) Adaptation fthe thylakoid membranes of pea chloroplasts to light intensities. I. Study on the distribution of chlorophyll-protein complexes. Photosyn Res **5:** 105–115
15. **Moran R** (1982) Formulae for determination of chlorophyllous pigments extracted with *N,N*-dimethylformamide. Plant Physiol **68:** 1376–1381
16. **Prioul J-L, Reyss A** (1987) Acclimation of ribulose bisphosphate carboxylase and mRNAs to changing irradiance in adult tobacco leaves. Plant Physiol **84:** 1238–1243
17. **Wild A, Hopfner M, Ruhle W, Richter M** (1986) Changes in stoichiometry of photosystem II components as an adaptive response to high-light and low-light conditions during growth. Z Naturforsch **41c:** 597–603