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The Effect of Light on the Formation of Chlorophyll-Protein Complexes in Oat Seedlings during Greening

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Various light intensity and light quality were treated to oat seedlings to investigate the effect of light on the chlorophyll accumulation and the formation of chlorophyll-protein complexes. The increase of total chlorophyll accumulation and Chl (chlorophyll) a/b ratio was promoted under H (high intensity) white light during oat chloroplast development when compared to L (low intensity) white light. Also H white light was more effective in the formation of chlorophyll-protein complexes associated with PSI, CCI and CCII than L white light. The seedlings grown in various light quality caused little changes in total chlorophyll and Chl a/b ratio when compared to those grown in L white light. The assembly of LHCII trimer was more affected by L white light treatment in the formation of chlorophyll-protein complexes than red light treatment. The effect of blue light on the relative composition of chlorophyll-protein complexes was similar to that of L white light. Particualrly, blue light was more effective in the synthesis of LHCII monomer than the other light quality at the early stage of greening. When compared to red light, blue light was more effective in the formation of LHCII monomer. These results suggest that light intensity is more effective in the increase of chlorophyll accumulation and Chl a/b ratio than light quality, and light quality may be an important factor for the regulation of the organization in the chlorophyll-protein complexes during greening.

Key words : chlorophyll-protein complexes, *Avena sativa* L., light intensity, light quality, greening

1. INTRODUCTION

Development of proplastids into etioplasts is induced by growing plants in the dark. Dark grown-seedlings contain the chloroplastic proteins RuBP-Case (ribulose 1,5-biphosphate carboxylase) and CF 1 (α-subunit of adenosine triphosphate synthetase), but lack chlorophylls, thylakoid membranes, light-harvesting chlorophyll a/b-binding protein (LHCP) of PSII (photosystem II), and the chlorophyll a-binding protein of reaction center P-700 of PSI (CPI) (Mayfield and Huff, 1986). Upon illumination, these etioplasts rapidly differentiate photosynthetically active chloroplasts, accumulating chlorophylls, thylakoids and new polypeptides including LHCP and CPI (Dietz and Bogorad, 1987). Fractionation of plastid proteins by native green gel electrophoresis, by which chlorophyll-protein complexes are solubilized and separated with minimum loss of noncovalently bound chlorophyll, is a very important method in the study of thylakoid membrane composition, organization and biogenesis (Thornber, 1986). Chlorophyll-protein complexes have been divided to three parts, nomenclaturing CPI, LHCP and free chlorophyll (Alberte *et al.*, 1972). Current green gel system, by which those are subdivided into 16~20 parts with very little release of free pigment, resolves multiple PSI-LHCI complexes, multiple PSII-LHCII complexes, four oligomeric LHCII complexes, several reaction center complexes, and a number of small complexes from chlorophyll-protein complexes (Allen and Staehelin, 1991).

Since photosynthetic activity of plants makes it possible to convert light energy into biologically usable energy, chemical potential, the light regimes which contain light intensity, quality and time of illumination play a dominant role in the photosyn-

thetic activity of higher plants. The composition and function of photosynthetic apparatus are affected by light environment. Chloroplasts in higher plants harvest light via two photosystem, classified PSI and PSII, as well as their associated light-harvesting complexes, LHCI and LHCII. Biosynthesis of LHCP is due to the increase in chlorophyll, particularly chlorophyll b is quantitatively incorporated into newly assembled LHCs under light conditions (Hoober *et al.*, 1990; White and Hoober, 1994). The amount of light gives rise to distinct structural and biochemical differences during chloroplast development (Leong and Anderson, 1984; Leong *et al.*, 19 85; Lechowiez *et al.*, 1986). High light intensity affects on the enhancement of CPa synthesis and the decline of light-harvesting complexes synthesis (Torre and Burkey, 1990). The conversion of high light intensity to low light intensity leads to higher levels of light-harvesting complex of PSII apoprotein than high light intensity, and these results give rise to an increase in chlorophyll a and b (Sukenik *et al.*, 1987; Sukenik *et al.*, 1989; Sukenik *et al.*, 1990). There are significant differences in the relative distribution of chlorophyll-protein complexes and in the ultrastructure of chloroplast when the plants are grown under different light quality conditions. The seedlings grown in yellow light have higher PSI complexes and lower PSII complexes than those grown in red light (Glick *et al.*, 1985; Melis *et al.*, 1985; Deng *et al.*, 1989). It has been reported that the seedlings grown in blue light exhibit higher levels of chlorophyll a to chlorophyll b ratios and prenylquinone contents, and lower levels of xanthophyll to carotene ratios than those grown in red light. These changes also induce morphological differences in the ultrastructure of chloroplast (Leong *et al.*, 1985). However, the effect of light intensity and quality in chloroplast during greening are not fully understood. Also it is not known whether the organization of chlorophyll proteins during adaptation to different light quality environments is a general mechanism in higher plants.

In the present study, we investigated the effect of light on the formation of chlorophyll-protein complexes in greening of oat seedlings, and whether the adaptive changes observed in oat seedlings reflect a general mechanism that allows plants to adapt to light quality environments. We have, therefore, analyzed oat seedlings grown under blue, green and red light in the biochemical levels, and compared them with white light-grown plants.

2. MATERIALS AND METHODS

2.1. Plant material

The oat seeds (*Avena sativa* L.) were soaked in running tap water for 4 h and planted on the pot, and grown in growth chamber at 28 ± 1℃ with 70 % humidity under dark condition for 5 days. And the seedlings were grown under the continuous irradiation of different light quality for 48 h.

Light source was a white fluorescent light and shielded with selectively light enriched acrylfilters for red, green, and blue light. Light intensities used for growth of oat seedlings were 491 erg $cm^{-2}sec^{-1}$ for high intensity (H) of white light, 163 erg cm^{-2} sec^{-1} for low intensity (L) of white light, 47 erg $cm^{-2}sec^{-1}$ for red light, 50 erg $cm^{-2}sec^{-1}$ for green light and 65 erg $cm^{-2}sec^{-1}$ for blue light with radiometer (Metrologic, 60-535, USA), and emission spectra of light sources obtained with optical multichannel analyzer (EG & G PARU, 1460, USA) were described in Lee *et al.* (1995).

2.2. Thylakoid membrane isolation

For the isolation of thylakoid membranes, the seedlings were homogenized with Waring blender in homogenization buffer consisted of 50 mM HEPES (pH 7.6), 0.3 M sorbitol, 10 mM NaCl and 5 mM $MgCl_2$. To remove cellular debris the homogenate was filtered through four layers of cheesecloth, and the filtrate was centrifuged at 350 g for 10 min. The supernatant was pelleted at 5,000 g for 10 min. The membrane pellet was washed twice in washing buffer consisted of 50 mM HEPES (pH 7. 6), 0.1 M sorbitol, 10 mM NaCl and 5 mM $MgCl_2$. The resulting pellet was resuspended in a small

volume of the same buffer with 10% glycerol, aliquoted and frozen at −80℃. All procedures of thylakoid membrane isolation were performed at 4℃.

2.3. Thylakoid membrane solubilization

For native green gel electrophoresis thylakoid membrane was washed twice in 2 mM Tris-maleate (pH 7.0), and the washed pellet was resuspended in solubilization buffer containing 2 mM Tris-maleate (pH 7.0), 10% glycerol, 0.45% octyl glucoside, 0.45% decyl maltoside, and 0.1% LDS (lithium dodecyl sulfate). Solubilization buffer was added to give a ratio of total nonionic detergent to chlorophyll of 20 : 1 (w/w). Samples were incubated on ice for 30 min and centrifuged at 15,000 g for 10 min to remove insoluble materials.

2.4. Native green gel electrophoresis

Native green gel was consisted of a stacking gel containing 5% acrylamide, 25 mM Tris-HCl (pH 6.3), 50 mM glycine and 10% glycerol, and a resolving gel containing 8% acrylamide, 25 mM Tris-HCl (pH 8.3), 50 mM glycine and 10% glycerol. The acrylamide/bisacrylamide ratio was 100 : 1. The gel was polymerized by adding 0.05% ammonium persulfate and 0.05% TEMED (tetramethylethylenediamine). The electrode buffer contained 25 mM Tris, 192 mM glycine (pH 8.3), and 0.1% SDS (sodium dodecyl sulfate) as described by Allen and Staehelin (1991). After the gel was prerun at 10 mA for 1 h in cold chamber, samples (about 13.5 μg chlorophyll) were loaded onto the gel. The amount of chlorophyll was determined with spectrophotometer (Shimadzu, UV 240, Japan) according to the method of Lichtenthaler (1987). The gel was electrophorized at 10 mA constant current, 4℃ for 3 h.

2.5. Denaturing SDS-PAGE

For two-dimensional gels, gel slices were excised from native green gel lanes, incubated for 15 min at 55℃ in solubilization solution containing 25 mM Tris-HCl (pH 6.3), 50 mM glycine, 2% SDS, 2% β-mercaptoethanol, and 10% glycerol. Gel slices treated with the solution were loaded directly onto the gel of 12% polyacrylamide. PAGE (polyacrylamide gel electrophoresis) in the presence of SDS was performed as described by Laemmli (1970).

2.6. Densitometry scanning

Gel lanes of a native green gel were scanned using a TLC scanner (Shimadzu, CS-930, Japan). The measuring wavelength of the densitometer was 675 nm. Peak areas for each green band in the lane were measured and represented as a percentage of the total chlorophyll in chlorophyll-protein complexes excluding free pigment. The values for each green band were the average of independent three experiments.

3. RESULTS

3.1 Changes in the contents of chlorophyll

Oat seedlings were grown in H white light for 48 h and changes in the contents of chlorophylls from the seedlings were shown in Fig. 1. White

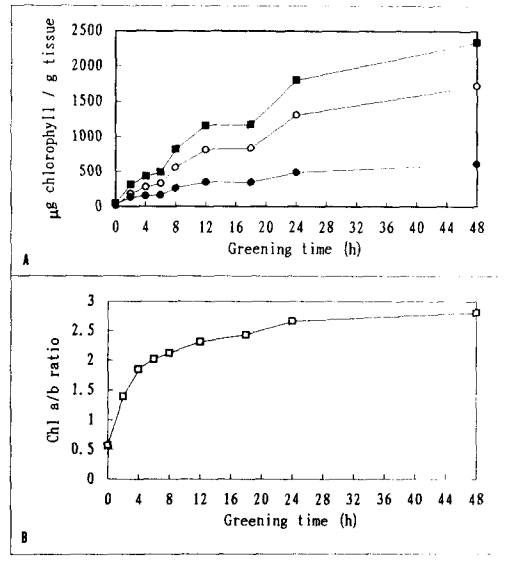

Fig. 1. Changes in chlorophyll contents (A) and Chl a/b ratios (B) from oat seedlings grown in high intensity of continuous white light for 48 h. The values are the average of three independent experiments. (A) −○−, chlorophyll a; −●−, chlorophyll b; −■−, total chlorophyll; (B) □−, Chl a/b ratio.

Table 1. Changes in chlorophyll contents and Chl a/b ratios from oat seedlings grown in continuous white light (low intensity) for 48 h

μg chlorophyll/g fresh weight

Duration of irradiation (h)	chlorophyll a	chlorophyll b	total chlorophyll	Chl a/b
0	14[a)]	25	39	0.57
2	142	186	328	0.76
4	224	245	469	0.91
6	306	316	622	0.97
8	528	332	860	1.59
12	649	369	1,018	1.76
18	773	393	1,166	1.97
24	910	442	1,352	2.06
48	1,052	501	1,553	2.10

[a)] The values are the average of three independent experiments.

Table 2. Changes in chlorophyll contents and Chl a/b ratios from oat seedlings grown in continuous red light for 48 h

μg chlorophyll/g fresh weight

Duration of irradiation (h)	chlorophyll a	chlorophyll b	total chlorophyll	Chl a/b
0	14[a)]	25	39	0.57
2	117	200	317	0.59
4	166	241	407	0.69
6	220	289	509	0.76
8	242	297	539	0.82
12	308	318	626	0.97
18	396	367	763	1.08
24	537	462	999	1.16
48	876	641	1,517	1.37

[a)] The values are the average of three independent experiments.

Table 3. Changes in chlorophyll contents and Chl a/b ratios from oat seedlings grown in continuous blue light for 48 h

μg chlorophyll/g fresh weight

Duration of irradiation (h)	chlorophyll a	chlorophyll b	total chlorophyll	Chl a/b
0	14[a)]	25	39	0.57
2	117	178	295	0.66
4	241	314	555	0.77
6	289	343	632	0.84
8	332	333	665	1.00
12	423	405	828	1.05
18	486	422	908	1.15
24	571	463	1,034	1.24
48	711	531	1,242	1.34

[a)] The values are the average of three independent experiments.

Table 4. Changes in chlorophyll contents and Chl a/b ratios from oat seedlings grown in continuous green light for 48 h

Duration of irradiation (h)	chlorophyll a	chlorophyll b	total chlorophyll	Chl a/b
			μg chlorophyll/g fresh weight	
0	14[a)]	25	39	0.57
2	133	242	375	0.55
4	181	288	469	0.63
6	208	287	495	0.73
8	242	341	583	0.71
12	331	387	718	0.86
18	522	490	1,012	1.06
24	654	553	1,207	1.19
48	859	648	1,506	1.33

[a)] The values are the average of three independent experiments.

light with the high intensity of 491 erg cm^{-2} sec^{-1} was used as a control. In oat seedlings grown in H white light, the contents of total chlorophyll were gradually increased along with greening period, thus those of total chlorophyll were given rise to about 58.5-fold increase during greening period investigated. When compared to the seedlings grown in H white light, those grown in L white light showed low level in the amount of total chlorophyll (Table 1). Light intensity, therefore, was a significant factor on the chlorophyll synthesis of chloroplast from oat seedlings. The seedlings grown in selectively enriched light quality for 48 h caused little changes in the accumulation of total chlorophyll when compared to those grown in L white light (Tables 2, 3 and 4).

Chl a/b ratio was an important parameter to measure light acclimation in oat seedlings. Chl a/b ratios of the light-adapted oat seedlings ranged from 0.57 to 2.81 (Fig. 1, Tables 1-4). The seedlings developed under H white light for 48 h had significantly higher Chl a/b ratios than those developed under L white light, while those grown in L white light had slightly higher Chl a/b ratios when compared to those grown in different light quality conditions. These results agree with those found previously in barley seedlings where high intensity seems to be more important factor to lead not only high Chl a/b ratios but also total chlorophyll synthesis enhancement (Torre and Burkey, 1990).

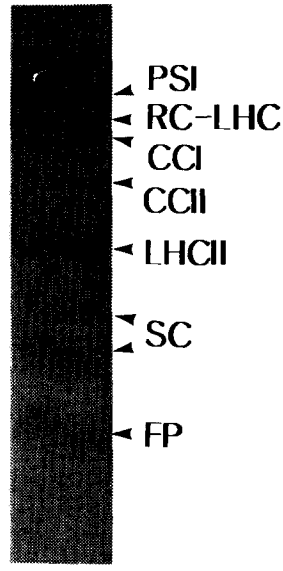

Fig. 2. Native green gel after electrophoresis of chloroplast from oat seedlings. Letters on the right-hand side indicate the designation of chlorophyll-protein complexes resolved. PSI, photosystem I complex; RC-LHC, reaction center-light harvesting complex; CCI, core complex of PSI; CCII, core complex of PSII of PSII; LHCII, trimeric form of the main light harvesting antenna PSII; SC, small complex; FP, free pigment.

3.2. Relative distribution of chlorophyll-protein complexes

The light-adapted oat thylakoids were solubilized with nonionic detergents/chlorophyll weight of 20

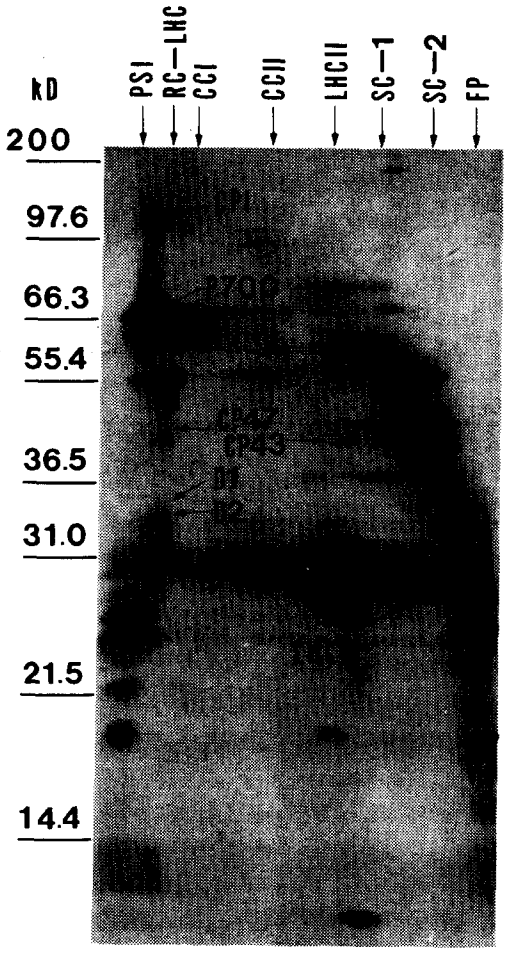

Fig. 3. Two dimensional gel in which oat chlorophyll-protein complexes have been separated on a native green gel in the first dimension, and on a full denaturing SDS-PAGE gel in the second dimension. Positions of selected green bands are indicated along the top of the gel. Silver-stained gel. The standard used were myosin (200 KD), phosphorylase (97.6 KD), BSA (66.3 KD), glutamic dehydrogenase (55.4 KD), lactate dehydrogenase (36.5 KD), carbonic anhydrase (31 KD), trypsin inhibitor (21.5 KD), and lysozyme (14.4 KD). P 700, P700 apoproteins; CP47/43, internal chlorophyll a-binding of PSII; D1/D 2, core apoproteins; LHCII*, apoproteins of LHCII.

: 1 and subjected to native green gel electrophoresis (Fig. 2). Eight main chlorophyll-containing bands were resolved, and they were classified in order of increasing mobility : PSI, RC (reaction center)-LHC, CCI (core complex of PSI), CCII (core complex of PSII), two bands of SC (small complex, SC-1 and SC-2), and free pigment. The band patterns were identified by their previously characterized spectral properties (Allen and Staehelin, 1991). Two of these complexes were associated with PSI : PSI complex and CCI, whereas four of these complexes were associated with PSII : CCII, LHCII and two bands of SC. Relative distribution of protein subunits in the different chlorophyll-protein complexes were quantitated after SDS-PAGE of the complexes in a second dimension (Fig. 3). PSI complexes were most easily seen in the second dimensional electrophoresis by the presence of P700 apoproteins, LHCI and the smaller PSI subunits. CCI associated with PSI contained a number of core complexes of PSI. CCII was visible on the second dimension gel by the presence of CP43 and CP47 apoproteins as well as D1 and D2 polypeptides. LHCII was preserved as the trimeric form of the main light harvesting antennae of PSII. SC contained a number of complexes, most of which appeared to be partially dissociated PSII components containing LHCII monomers.

3.3. Formation of chlorophyll-protein complexes during greening

The changes of relative distribution of chlorophyll-protein complexes in the oat seedlings grown in the continuous illumination of H white light for 48 h were shown in Fig. 4. The thylakoid membranes from dark-grown plants (0 h) could not be completely solubilized by the surfactant solution used. However, samples from the seedlings illuminated for 2 h under continuous light were resolved. At 4 h illumination, the dominant pigmented band was that of CCII. The accumulation of PSI holocomplexes containing LHCI apoproteins appeared to occur at 4 h and developed gradually throughout the greening period. The paint band of SC which seemed to contain LHCII monomer appeared at 4 h illumi-

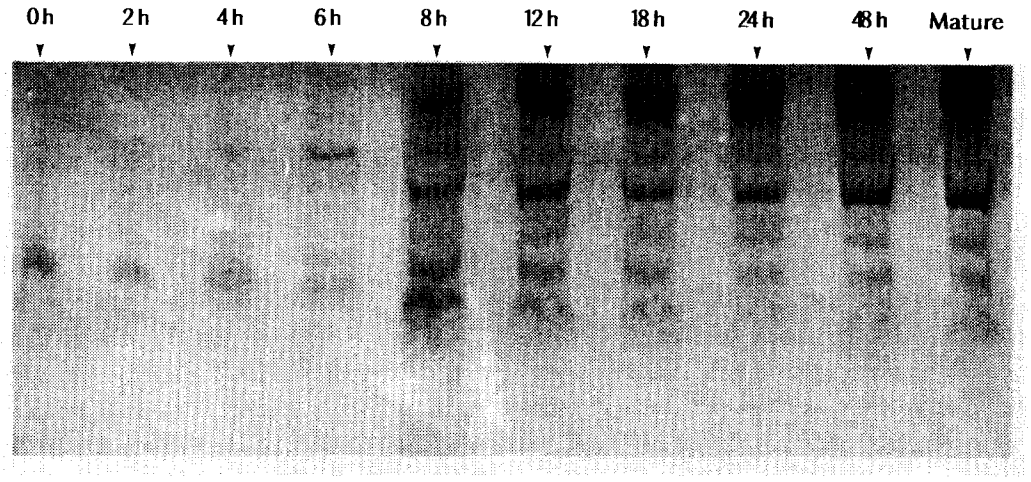

Fig. 4. Changes in the formation of chlorophyll-protein complexes from oat seedlings grown in high intensity of continuous white light for the indicated times. Thylakoid membranes (13.5 μg of chlorophyll) were solubilized with octyl glucoside, decyl maltoside and lithium dodecyl sulfate.

nation. As the accumulation of SC progressed, there was a steady increase in the amount of LHCII which seemed to be a trimer from the first 6 h of greening. The fact supports that LHCII apoproteins aggregated into trimer seems initially to accumulate in the thylakoid membranes as monomeric pigment proteins during development of the chloroplast (Dreyfuss and Thornber, 1994a). After 8 h of continuous illumination, chlorophyll-protein complexes of oat seedlings could be completely separated into eight main green bands. The relative distribution of the complexes in the seedlings grown for 48 h was similar to those in the mature seedlings. The relative percentages of each complexes in those grown for 48 h were 32% of PSI, 7% of RC-LHC, 5% of CCI, 8% of CCII, 23% of LHCII, and 25% of SC excluding free pigment.

3.4. Effect of light on the formation of chlorophyll-protein complexes

Oat seedlings grown in L white light for 48 h induced the quantitative changes in chlorophyll-pro-

Table 5. Changes in the relative amount of chlorophyll in chlorophyll-protein complexes from oat seedlings grown in white light (low intensity) for 48 h

Duration of irradiation (h)	Percentage of chlorophyll in chlorophyll-protein complexes						
	PSI	RC-LHC	CCI	CCII	LHCII	SC-1	SC-2
0	–	–	–	–	–	–	–
2	–	–	–	100	–	–	–
4	5.83	–	–	24.85	24.73	–	44.59
6	13.11	–	–	14.19	21.44	11.88	39.39
8	26.45	–	1.51	5.51	23.90	14.37	28.27
12	25.10	2.02	0.79	7.02	28.43	10.31	26.33
18	29.95	7.06	1.22	6.66	26.39	9.53	19.19
24	24.85	9.18	2.71	5.97	28.47	11.15	17.65
48	29.81	6.64	3.66	6.00	28.23	10.36	15.29

tein complexes when compared to those grown in H white light for 48 h (Fig. 4 and Table 5). The seedlings grown in H white light showed lower level in the chlorophylls associated with LHCII and higher level in the amount of PSI and CCII than those grown in L white light. The chlorophylls associated with CCI also had slightly enhancement in those grown in H white light compared to those grown in L white light. The fact suggests that light intensity affects the amount of chlorophyll associated with core complexes of PSI and PSII, although light intensity does not affect the amount of chlorophylls associated with PSI (Leong and Anderson, 1984).

The amount of chlorophyll associated with each complex showed differences between the seedlings grown in L white light and those grown in red light as shown in Tables 5 and 6. Chloroplasts adapted to red light showed increased levels of chlorophylls associated with RC-LHC and CCII, and decreased levels of chlorophylls associated with LHCII and SC containing LHCII monomer of PSII as compared with those grown in L white light. Oat seedlings grown in blue light for 48 h had similar relative chlorophyll contents in the chlorophyll-protein complexes when compared to those grown in L white light for 48 h (Table 7). However, SC containing LHCII monomer appeared alone in 2 h-grown

Table 6. Changes in the relative amount of chlorophyll in chlorophyll-protein complexes from oat seedlings grown in red light for 48 h

Duration of irradiation (h)	Percentage of chlorophyll in chlorophyll-protein complexes						
	PSI	RC-LHC	CCI	CCII	LHCII	SC-1	SC-2
0	–	–	–	–	–	–	–
2	–	–	–	–	–	–	–
4	10.96	–	–	–	27.40	–	61.64
6	17.28	–	–	7.74	32.34	–	42.64
8	18.47	–	1.80	8.49	29.77	3.33	38.13
12	15.61	–	4.23	6.16	17.76	9.97	46.26
18	27.16	1.09	1.72	8.29	30.07	10.88	20.80
24	28.40	2.90	1.74	8.00	31.80	10.10	17.10
48	30.10	7.60	4.10	7.30	27.60	9.00	14.30

Table 7. Changes in the relative amount of chlorophyll in chlorophyll-protein complexes from oat seedlings grown in blue light for 48 h

Duration of irradiation (h)	Percentage of chlorophyll in chlorophyll-protein complexes						
	PSI	RC-LHC	CCI	CCII	LHCII	SC-1	SC-2
0	–	–	–	–	–	–	–
2	–	–	–	–	–	–	100
4	33.09	–	–	–	37.89	–	29.02
6	24.35	–	2.46	9.57	21.56	–	42.06
8	23.37	–	2.42	8.23	27.20	8.15	30.61
12	26.22	–	1.77	6.08	25.74	10.37	29.82
18	28.58	3.25	2.83	6.73	24.35	11.42	22.84
24	29.71	4.93	3.53	5.94	24.14	11.86	19.89
48	29.54	6.67	4.63	5.18	27.62	10.48	15.88

Table 8. Changes in the relative amount of chlorophyll in chlorophyll-protein complexes from oat seedlings grown in green light for 48 h

Duration of irradiation (h)	Percentage of chlorophyll in chlorophyll-protein complexes						
	PSI	RC-LHC	CCI	CCII	LHCII	SC-1	SC-2
0	–	–	–	–	–	–	–
2	–	–	–	71.75	–	–	28.25
4	12.21	–	–	15.13	24.93	–	47.74
6	17.00	–	–	13.55	22.57	12.22	34.66
8	17.27	–	2.91	9.21	25.29	9.20	36.12
12	24.79	4.93	2.98	5.95	26.09	10.19	25.07
18	28.78	4.92	2.38	5.13	25.38	10.66	22.76
24	25.66	5.81	6.12	6.09	24.69	11.88	19.76
48	26.01	9.56	10.94	4.39	24.58	9.16	15.36

seedlings under blue light. This result suggests that blue light appears to be more effective in the accumulation of LHCII monomer than L white and red light conditions at the early stage of greening. When compared to the seedlings grown in red light, those grown in blue light led slightly higher chlorophyll contents in SC. These results agree with those found previously with *Asplenium australasicum* seedlings where blue light was more effective in the synthesis of LHCP than red light (Leong *et al.*, 1985). On the other hand, chloroplasts from the seedlings grown in green light were enriched in RC-LHC and CCI when compared to those from the seedlings grown in L white light (Table 8).

4. DISCUSSION

The light-adapted oat seedlings were resolved to eight main green bands by native green gel electrophoresis (Fig. 2). The green band patterns resembled those reported by others using *Arabidopsis* and barley (Allen and Staehelin, 1991; Peter and Thornber, 1991a), and the bands were classified in order of increasing mobility to PSI, RC-LHC, CCI, CCII, LHCII, two bands of SC (SC-1 and SC-2), and free pigment. To better understand relative distribution of the protein subunits in the complexes, gel lanes obtained by native green gel were resolved by SDS-PAGE of the complexes in a second dimension (Fig. 3). PSI holocomplexes contained P 700 apoproteins, LHCI apoproteins and the smaller PSI subunits. This fact seems to be consistent with some reports (Dreyfuss and Thornber, 1994b). CCI associated with PSI contained a number of core complexes of PSI. CCII contained CP43/CP47 and D1 /D2 apoproteins and migrated close to the LHCII trimer, and these results can be also seen in some of other plants (Peter and Thornber, 1991b). LHCII was seemed to be an aggregate of trimeric pigment-protein units, and it has been known to be composed of at least three types of protein subunits (24~29 kD) of slightly different primary structure (Butler and *Kühlbrandt*, 1988; *Kühlbrandt* and Wang, 1991; Peter and Thornber, 1991c). SC contained a number of complexes, most of which appeared to be partially dissociated PSII components containing LHCII monomer.

Use of native green gel electrophoresis led to the fractionation from chlorophyll-protein complexes of greening chloroplasts with negligible displacement of chlorophylls in the complexes. The status of assembly of the various chlorophyll-protein complexes was easily represented throughout the maturation of the chloroplast (Fig. 4). PSI holocomplex containing LHCI apoproteins appeared to accumulate after 4 h of continuous illumination and the accumulation of PSI holocomplex developed gradually throughout the entire greening period. The results agree with the observations using barley

seedlings (Dreyfuss and Thornber, 1994a). After the accumulation of SC, seemed to contain LHCII monomer, at 4 h illumination, there was a steady accumulation of LHCII seemed to be a trimeric pigmented-protein complexes from the first 6 h of greening. It is suggested, therefore, that pigmented-monomeric protein complexes formed early during light-adapted development of chloroplast are reorganized into the formation of trimeric LHCII complexes that form the bulk of the antenna complex of PSII, and these results agree with those found previously (Hobe *et al.*, 1994; Dreyfuss and Thornber, 1994a). The dominant pigmented band at 4 h illumination was that of CCII, and the amount of CCII decreased during the greening period in the relative distribution of various pigmented-protein complexes. The various chlorophyll-protein complexes were completely formed after 8 h of continuous illumination.

Light intensity affects chlorophyll content, Chl a /b ratio and the formation of chlorophyll-protein complexes in oat seedlings. The seedlings grown in H white light had higher total chlorophyll contents and Chl a/b ratios when compared to those grown in L white light (Fig. 1 and Table 1), suggesting that light intensity may be an important factor to induce the increase of Chl a/b ratio and chlorophyll accumulation in oat seedlings. These results are similar to those found previously, where chlorophyll content and Chl a/b ratio are higher in the barley leaves grown in high intensity of light compared to those grown in low intensity of light (Torre and Burkey, 1990). Other plant species also show the similar results in the chlorophyll content and Chl a/b ratio (Davies *et al.*, 1986; Lee and Whitmarsh, 1989). From the results for Chl a/b ratio it could be suggested that light intensity induced differences in the distribution of chlorophyll between chlorophyll-protein complexes. High intensity of light induced higher levels in Chl a/b ratio as well as the amount of PSI complex, CCI and CCII, and lower levels in the amount of LHCII trimer in the chlorophyll-protein complexes from oat seedlings than low intensity of light (Fig. 4 and Table 5). From these results it is suggested that light intensity plays an important role in the synthesis of chlorophylls associated with PSI, CCI and CCII, contrary to the findings that light intensity does not affect the amount of chlorophylls associated with PSI (Leong and Anderson, 1984).

The oat seedlings grown in various light quality conditions (red, blue and green light) caused little changes in the amount of total chlorophyll and Chl a/b ratio during greening when compared to those grown in L white light (Tables 1-4), suggesting that light intensity is more effective in the increase of chlorophyll accumulation and Chl a/b ratio than light quality. And these results seem to be consistent with those found previously (Torre and Burkey, 1990). There are significant differences in the relative distribution of chlorophyll-protein complexes of thylakoids adapted to different light quality conditions. Red light adaptation led to higher levels in chlorophylls associated with RC-LHC and CCII, and lower levels in chlorophylls associated with LHCII trimer and SC containing LHCII monomer than L white light adaptation did (Tables 5 and 6). These results are similar to some reports that thylakoids from corn leaves adapted to red light have lower amount of LHCP than those adapted to white light (Eskins *et al.*, 1985). Other plant species also show the similar composition in chlorophyll-protein complexes (Leong *et al.*, 1985). The previous studies on red or white light effect, however, reported that light quality caused a reorganization of the components of chlorophyll-protein complexes such that plants grown in white light were enriched in PSI complexes, while those grown in red light were enriched in PSII complexes (Glick *et al.*, 19 86; Deng *et al.*, 1989). It is suggested, therefore, that the effect of light quality on the composition of chlorophyll-protein complexes during chloroplast development are an important factor, but not the same for all plant species. The effect of blue light was similar to that of L white light on the relative distribution of chlorophyll-protein complexes du-

ring oat chloroplast development (Tables 5 and 7). However, SC containing LHCII monomer was appeared alone in 2 h-illuminated seedlings under blue light compared to different light quality conditions. The facts suggest that blue light may be more effective in the biosynthesis of LHCII monomer than other light quality at the early stage of greening. It agrees with the reports for pea plants, where blue light induces specifically pigment-free components of LHCP during the early stage of chloroplast development (Adamska *et al.*, 1992). When compared to the seedlings grown in red light, those adapted to blue light had slightly higher chlorophylls associated with SC. This is in agreement with prior results obtained from similar studies with *Asplenium australasicum* (Leong *et al.*, 1985). From these results it is suggested that blue light is more effective in the synthesis of LHCII monomer than red light. Green light adaptation was more effective in the chlorophylls associated with RC-LHC and CCI than white light adaptation during oat chloroplast development (Tables 5 and 8).

The results of the present study have established the light-induced assembly of chlorophyll-protein complexes during greening and suggested the important role of light intensity and quality that control the formation of chlorophyll-protein complexes during greening. And further studies are necessary to clarify the molecular mechanism that allows chloroplasts to adapt to different light quality, because light quality affects structural and functional adaptation in the thylakoids and the changes are attributed by the plastid mRNA encoding the proteins for the two photosystem.

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녹화중 귀리 유식물의 엽록소-단백질 복합체 형성에 미치는 광선의 효과

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백색광 및 여러 파장의 광선(적색, 청색, 그리고 녹색광)이 녹화중인 귀리 유식물의 엽록소 함량 변화 및 엽록소-단백질 복합체 형성에 미치는 효과를 조사하였다. 높은 광도의 백색광은 낮은 광도의 백색광에 비하여 귀리 유식물의 전체 엽록소 함량 및 엽록소 a/b 비율의 증가에 효과적이었다. 또한 엽록소-단백질 복합체의 형성에 있어서 높은 광도의 백색광은 낮은 광도의 백색광에 비하여 PSI, CCI, 및 CCII의 형성을 효과적으로 촉진하였다. 한편, 여러 파장의 광선하에서 생장한 귀리 유식물은 유사한 광도를 가진 백색광하에서 생장한 유식물에 비하여 엽록소의 축적 및 엽록소 a/b 비율에 큰 영향을 받지 않았다. 적색광은 귀리 유식물의 엽록소-단백질 복합체의 형성에 있어서 백색광에 비하여 LHCII trimer 형성에 효과적이지 못하였으나, 청색광은 백색광과 유사한 효과를 나타내었다. 청색광과 적색광을 비교하였을 때, 청색광은 적색광보다 LHCII monomer의 형성에 효과적이었다. 이와 같은 결과에서 엽록체의 발달과정동안 광도의 효과가 광질의 효과보다 엽록소 축적 및 엽록소 a/b 비율의 증가에 중요한 인자로 작용하며, 여러 파장의 광선은 엽록체-단백질 복합체의 조성을 조절하는데 중요한 인자인 것으로 사료된다.