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Assembly of the Light-Harvesting Complexes During Plastid Development

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The light-induced assembly of functional components of the thylakoid membrane has been followed during the biogenesis of the wheat plastid. The levels of light-harvesting complex (LHC) apoproteins in the 30- to 18-kDa region increase rapidly upon exposure to continuous light (CL). The newly synthesized LHC II apoproteins appear to be present predominantly in the monomeric forms. Upon further greening the LHC II apoproteins are increasingly located in LHC II oligomeric forms. At the same time, during the first times of greening, the bands in the 20.5- to 19- and 17.5- to 15.5-kDa regions related to early light-induced proteins (ELIP) have been observed. Low-temperature fluorescence spectra (77 K) of chloroplasts isolated from intermittent light (IML)-grown leaves display a main emission band at 687 nm and a small one at 727 nm. As greening proceeds, the 727-nm peak gradually shifts to longer wavelengths and increases in intensity relative to the 687-nm emission peak. By 6 h, in seedlings transferred to CL, the fluorescence spectrum is characterized with bands at 687, 696, and 740 nm (main). The increase in fluorescence intensity at 740 nm takes place due to restoration of the energy transfer to the Chl a form at 710–712 nm from LHC I synthesized during CL.

KEY WORDS: Wheat plastids; greening; early light-induced proteins; biogenesis; intermittent light.

INTRODUCTION

PS I and PS II of higher plant thylakoid membranes include CC[3] containing the photochemical reaction center and an LHC, the function of which is to absorb photons and to transfer excitation energy to the CC [1]. Angiosperms produce etiolated seedlings when exposed to prolonged darkness [2]. Growth of etiolated plants in intermittent light arrests plastid development at the stage of the protochloroplast. Protochloroplasts synthesize Chl a and carotenoids selectively and are essentially devoid of Chl b [3]. The formation of thylakoid membranes during this greening process occurs with the sequential appearance of PS I, followed by PS II, intersystem electron-transport components, and, finally, the assembly of LHC II and the PS I light-harvesting complex. In plants exposed to intermittent light, or in plants transferred to darkness after brief preexposure to continuous light (i.e., under conditions where Chl synthesis is stopped), preaccumulated LHC II apoproteins are degraded because of rapid turnover of their apoproteins in the absence of stabilization by the photosysthetic pigments, while the reaction center components continue to be synthesized [4]. Thus when Chl accumulation is low, LHC apoproteins cannot form Chl–protein complexes [5–7]. In the present paper low-temperature fluorescence spectroscopy and high-resolution SDS-PAGE are employed to examine the assem-

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[3] *Abbreviations used*: CL, continuous light; CC, core complex; ELIP, early light-induced proteins; IML, intermittent light; LHC, light-harvesting complex; PS, photosystem.

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bly of light-harvesting complexes in greening wheat seedlings.

MATERIALS AND METHODS

Wheat (*Triticum durum* L.) seeds were germinated and grown in complete darkness in a growth chamber on filter paper moistured by water at 23 ± 2°C and 60–70% relative humidity for 5 days and then subjected to intermittent white-light flashes [2 min of light (40 $\mu Es^{-1}m^{-2}$), 118 min of darkness] for 3 days. They were then exposed to increasing periods (0, 2, 6, 12, and 36 h) of continuous white light (125 $\mu Es^{-1}m^{-2}$). Mature control plants were germinated and grown in a growth chamber for 8 days. Chloroplast isolation and thylakoid membrane precipitation were carried out according to Peter and Thornber [8]. The chlorophyll concentration was determinated spectrophotometrically in a 96% ethanol extract according to Inskeep and Bloom [9].

Thylakoid proteins were separated with SDS-PAGE according to Laemmli [10], as described in Ref. 11, using a 6% stacking gel and a 10 to 25% (w/v) acrylamide gradient in the separating gel (30.0:0.8 acrylamide/bisacrylamide) on plates of 1 × 160 × 180 mm. After electrophoresis the protein bands were visualized and stained for 30 min with a solution of 0.04% (w/v) Coomassie brilliant blue G-250 prepared on 3.5% perchloric acid. Molecular weight markers were purchased from Sigma. The gels were scanned on an Ultroscan-2202 laser densitometer (LKB, Sweden).

The spectral measurements were done using a double-beam Hitachi-557 spectrophotometer and an Hitachi-850 fluorescence spectrophotometer (Japan) as described previously [12].

RESULTS AND DISCUSSION

Figure 1 shows the density gradient electrophoresis in a 10–25% polyacrylamide gel with 0.1% SDS. As significant changes are not observed in the region of the PSI core (CPI), CPI apoprotein, α- and β-subunit CF_1 of the ATP synthase complex, and the core antenna of PS II (CP 47 and CP 43), attention was concentrated on polypeptides of the light-harvesting complex of PS I and II (LHC I and LHC II, respectively).

In samples from IML-grown plants (0 h; i.e., samples after a cycle of 2-min light, 118-min darkness), in the 30- to 18-kDa region there are some low-intensity bands, and at 2 h of exposure to CL their number and intensity tend to increase (see Fig. 1 and Table I). Then upon

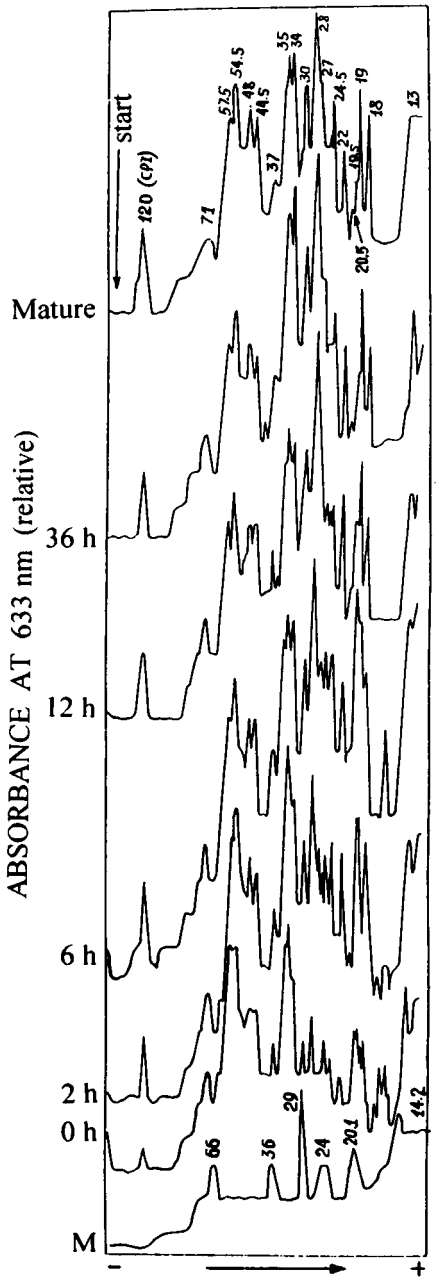

Fig. 1. Density patterns from gradient (10–25%) SDS-PAGE analysis of thylakoid membranes of IML-grown seedlings exposed to continuous illumination for 0 to 36 h and mature greenhouse-grown seedlings. Molecular mass standards (kDa) are marked below.

illumination to mature chloroplasts, the number of bands decreases, but the synthesis of the main proteins of antenna complexes (29, 28-, 24.5-, 22-, 19-, and 18-kDa proteins) increases. Hence it was concluded that the monomeric forms of light-harvesting Chl a/b proteins aggregate to form higher-order oligomeric forms. It may

Table I. LHC Proteins in the Region 30–18 kDa

	Molecular mass of proteins (kDa)							
Sample	29	28	27	25	24.5	22	19	18
0 h	+	+	−	+	Trace	+	+	Trace
2 h	+	+	Trace	+	+	+	+	+
6 h	+	+	+	+	+	+	+	+
12 h[a]	+	+	+	−	+	+	+	+

[a] After 12 h of illumination significant changes in the composition of key bands are not observed.

be suggested that this stage is the highest of LHC assembly in the developing chloroplast.

Special interest was focused on polypeptides discovered on SDS-PAGE in the 20.5- to 19- and 17.5- to 15.5-kDa regions. According to modern literature data these polypeptides are called "early light-induced proteins" [13,14]. As shown in Fig. 1, in samples from IML-grown plants (0-h samples) they form some bands. After 2 h of illumination on CL the number of bands related to these polypeptides decreases. At the same time the band in the 15-kDa region increases. The intensity of this band reaches a maximum level in 6 h and it has almost disappeared a few hours later on CL. For this reason they were christened early light-inducible proteins and it was proposed that they were involved in the etioplast-to-chloroplast transition. It seems that the proteins discovered by us from high-resolution gradient gel electrophoresis (ELIP) fulfills a certain function in the process of plastid development. And in the further growth and development of chloroplasts, they are not necessary and, possibly, are exposed to proteolysis by proteolitic enzymes in the cell. Their nature and function are unknown, but an involvement in pigment synthesis or in PS II repair after photoinhibition has been suggested [15]. On the another hand, in higher plants they seem to have a special role in photoprotection [16].

Figure 2 shows the fluorescence spectrum at 77 K for chloroplasts from IML-grown seedlings exposed to continuous illumination for various times (0–12 h) and from mature greenhouse-grown seedlings. The data on the appearance of the LHC I and LHC II subunits during light-driven biogenesis of the plastid were correlated with the appearance of the long-wavelength fluorescence band at 77 K, characteristic of LHC I biogenesis and its assembly with CC I. Low-temperature fluorescence spectra (77 K) of chloroplasts isolated from IML-grown leaves display a main emission band at 687 nm and a small peak at 727 nm, characteristic of the presence of PS II and CC I, respectively [17]. As greening proceeds after 2 h

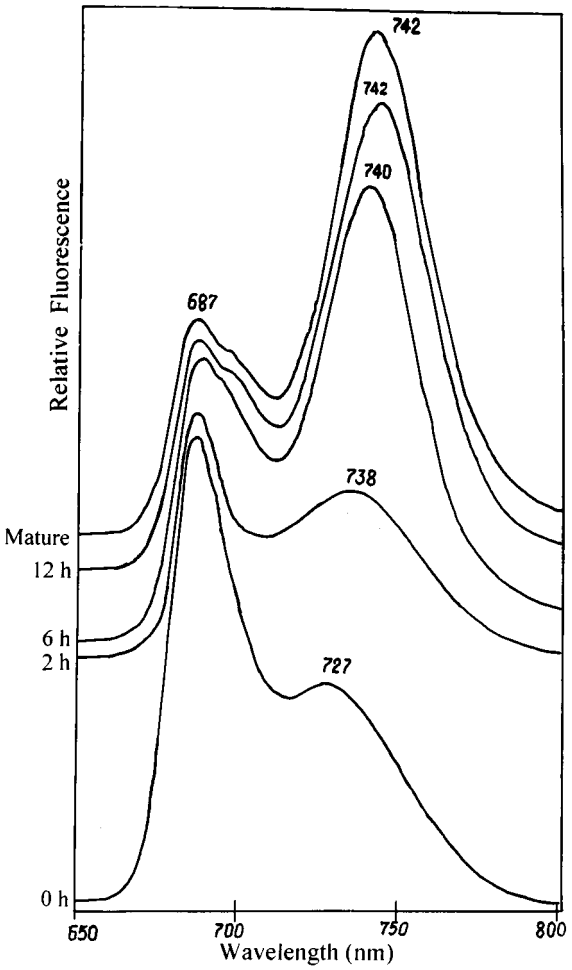

Fig. 2. Fluorescence emission spectra at 77 K obtained from chloroplasts of IML-grown wheat seedlings exposed to various periods of constant illumination and mature light-grown seedlings. Spectra were normalized to equivalent emissions at 687 nm.

the 727-nm peak, corresponding to CC of PS I, gradually shifts to longer wavelengths of 736–738 nm and increases in intensity relative to the 687-nm emission peak. By 6 h in seedlings transferred to CL this peak is at 740 nm, which clearly demonstrates the presence of completely synthesized PS I units. It was found that the fluorescence characteristics of chloroplasts greened for 12 h are similar to those of mature chloroplasts. The appearance of long-wavelength fluorescence emission at 740–742 nm during greening suggested that the peripheral antenna of PS I was synthesized when these plants were exposed to continuous illumination [5,6,18,19]. The addition of antennae to PS I could have occurred either by the addition of Chl to preexising protein sites or by the synthesis and insertion of new Chl–protein complexes. The changes in the proportion of the long-wavelength emission band probably

represent the decreased presence of CC I in the thylakoid membrane, because CC I is utilized in the formation of PS I [5,6].

Figure 3 shows the absorption spectra (A) and their fourth derivatives (A^{IV}) at 77 K of the chloroplasts from the seedlings for IML and for CL seedlings. In chloroplasts for IML seedlings, peaks of Chl a appeared at 660, 665 (shoulder), 669, 676, 682, 690, 696, 704, and 712 nm, and a small peak of Chl b at 648 nm. On the transfer of the seedlings from intermittent to continuous illumination in spectrum A^{IV} (77K), the intensity of the Chl b peak at 648 nm increases considerably, obviously corresponding to the synthesis under these conditions of LHC I and LHC II. At the same time a sharp fall takes place in the peak intensity at 682 nm relative to the first spectrum. The Chl a form at 710–712 nm is known to be responsible for the long-wavelength fluorescence band of the chloroplasts at 740 nm. It is important to note that the long-wavelength Chl a forms are present in the fourth derivatives of absorption spectra at 77 K of chloroplasts isolated from IML wheat seedlings. Therefore, the shift to the short-wavelength region with the heavy fall in the intensity of the long-wavelength fluorescence band at 77 K of the chloroplasts in IML (Fig. 2) cannot be explained by the absence of the longest-wave Chl a forms. Most probably in the chloroplasts in IML there is an absence of energy transfer from the light-harvesting complex to the Chl a form at 710–712 nm, and therefore, this form fluoresces weakly (Fig. 2). Thus with a switch of the seedlings from IML to CL a sharp rise takes place in the fluorescence intensity at 740 nm, which is due to restoration of the energy transfer to the Chl a form at 710–712 nm from LHC I synthesized during CL.

Our data may be interpreted within the framework of the concept of Tzinas and Akoyunoglou [20], according to which there is a competition of the polypeptides of the reaction center and LHC for the newly synthesized Chl a molecules. In the absence or lack of Chl b (and, consequently, LHC I and LHC II), the newly synthesized Chl a molecules are taken up to a greater degree by the polypeptides of the reaction center of PS I and PS II. In seedlings transferred from IML to CL, the newly synthe-

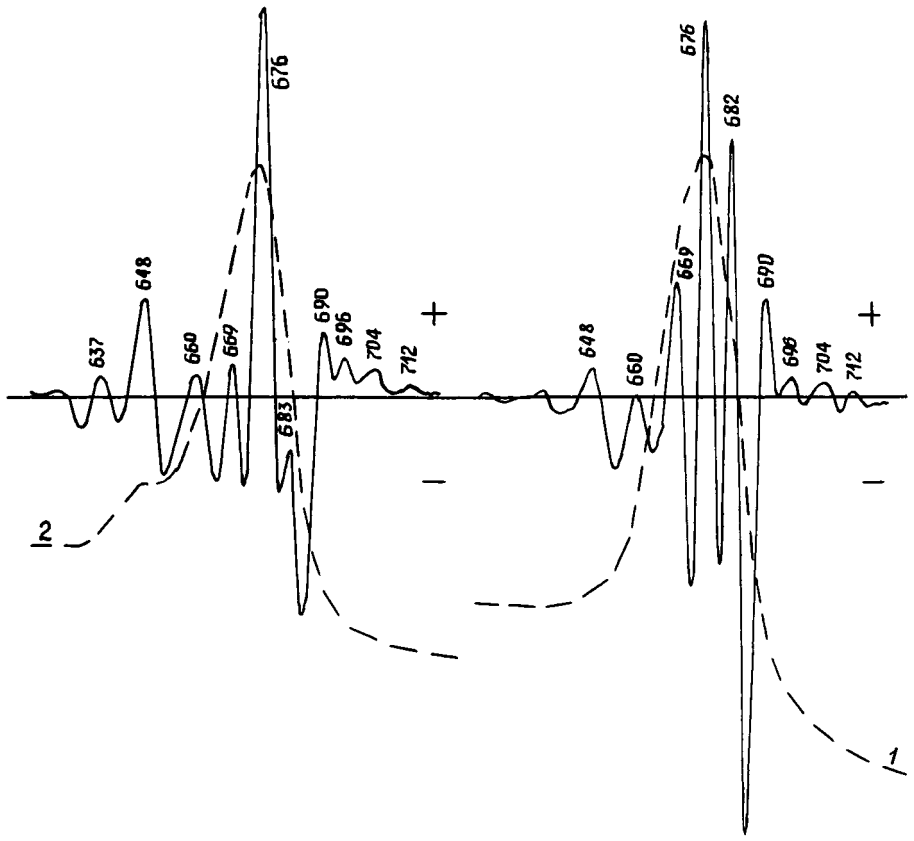

Fig. 3. Absorption spectra and their fourth derivatives at 77 K of the chloroplasts of wheat seedlings in IML (1) and after transfer to CL (2). Intervals of differentiation are $\Delta\lambda = 7$ and $\Delta\lambda = 6$, respectively.

sized Chl a molecules are taken up by the apoproteins of LHC and the number of reaction centers of Chl–protein complexes decreases.

The results obtained by us suggest that newly synthesized LHC apoproteins appear in the thylakoid membranes primarily as monomeric pigmented complexes that later assemble higher-order oligomeric forms. The appearance of the oligomeric forms of LHC is delayed in comparison to the accumulation of the monomeric LHC apoproteins. This delay may be due to the sequential pathway of assembly and may explain the longer time required to proceed beyond the pigmented monomeric form, or it may be due to a requirement for other factors for the assembly of the monomeric LHC into its oligomeric forms.

REFERENCES

1. J. P. Thornber (1986) in *Encycl. Plant Physiol.,* Vol. 19, Springer-Verlag, Berlin, pp. 98–142.
2. R. M. Leech (1984) in N. R. Baker and J. Barber *Photosynthesis: Chloroplast Biogenesis, Vol. 5*, Elsevier, Amsterdam, pp. 1–21.
3. J. H. Argyroudi-Akoyunoglou and G. Akoyunoglou (1979) *FEBS Lett.* **104**, 78–84.
4. M. Vivo and K. Kloppstech (1982) *Planta* **154**, 18–23.
5. B. W. Dreyfuss and J. P. Thornber (1994a) *Plant Physiol.* **106**, 829–839.
6. B. W. Dreyfuss and J. P. Thornber (1994b) *Plant Physiol.* **106**, 841–848.
7. A. N. Webler and N. R. Baker (1996) in D. K. Ort and C. F. Yokum (eds.), *Oxygenic Photosynthesis: The Light Reactions*, Kluwer Academic, Dordrecht, The Netherlands, pp. 41–58.
8. G. F. Peter and J. P. Thornber (1991) *J. Biol. Chem.* **266**, 16745–16754.
9. W. P. Inskeep and P. R. Bloom (1985) Extinction coefficients of chlorophyll a and b in N,N-dimethylformamide and 80% acetone. *Plant Physiol.* **77**, 483–485.
10. V. K. Laemmli (1970) Cleavage of structural proteins during the assembly of the head of bacteriofage T4. *Nature* **227**, 680–685.
11. J. A. Aliev, S. Yu. Suleimanov, I. M. Guseinova, M. A. Ismailov, and A. A. Asadov (1993) *Photosynthetica* **29**, 361–367.
12. A. A. Asadov, I. S. Zulfugarov, S. Yu. Suleimanov, and J. A. Aliev (1986) *Dokl. Akad. Nauk SSSR* **287**, 444–447 (Russian).
13. I. Adamska, K. Kloppstech, and I. Ohad (1993) *J. Biol. Chem.* **268**, 5438–5444.
14. M. Krol, M. D. Spangfort, N. P. Huner, G. Oquist, P. Gustafsson, and S. Jansson (1995) *Plant Physiol.* **107**, 873–883.
15. I. Adamska, K. Kloppstech, and I. Ohad (1992) *J. Biol. Chem.* **267**, 24732–24737.
16. G. Brittion (1993) in C. Sundqvist (ed.), *Pigment-Protein Complexes in Plastids: Synthesis and Assembly. Cell Biology: A Series of Monographs*, Academic Press, San Diego, CA, pp. 447–483.
17. S. Yu. Suleimanov, I. M. Guseinova, S. N. Novruzova, and J. A. Aliev (1998) in G. Garab (ed.), *Photosynthesis: Mechanisms and Effects. Proceedings of the XIth International Congress on Photosynthesis*, Kluwer Academic, Dordrecht, Vol. IV, pp. 3171–3174.
18. J. E. Mullet, J. J. Burke, and C. A. Arntzen (1980a) *Plant Physiol.* **65**, 814–822.
19. J. E. Mullet, J. J. Burke, and C. A. Arntzen (1980b). *Plant Physiol.* **65**, 823–827.
20. G. Tzinas and J. H. Argyroudi-Akoyunoglou (1988) *FEBS Lett.* **229**, 135–141.