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Reorganization of Thylakoid Components during Chloroplast Development in Higher Plants after Transfer to Darkness[1]

CHANGES IN PHOTOSYSTEM I UNIT COMPONENTS, AND IN CYTOCHROMES

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ABSTRACT

It was shown earlier that in etiolated bean (*Phaseolus vulgaris*, var. red kidney) leaves exposed to continuous light for a short time and then transferred to darkness a reorganization of their photosystem II (PSII) unit components occurs. This reorganization involves disorganization of the light-harvesting complex of PSII (LHC-II), destruction of its chlorophyll *b* and the 25 kilodalton polypeptide, and reuse of its chlorophyll *a* for the formation of additional, small in size, PSII units (Argyroudi-Akoyunoglou, Akoyunoglou, Kalosakas, Akoyunoglou 1982 Plant Physiol 70: 1242–1248). The present study further shows that parallel to the PSII unit reorganization a reorganization of the PSI unit components also occurs: upon transfer to darkness the 24, 23, and 21 kilodalton polypeptides, components of the light-harvesting complex of PSI (LHC-I), are decreased, the 69 kilodalton polypeptide, component of the chlorophyll *a*-rich P700-protein complex (CPI), is increased and new small-sized PSI units are formed. Concomitantly, the cytochrome *f*/chlorophyll and the cytochrome *b*/chlorophyll ratios are gradually increased. This suggests that the concentration of the electron transport components is also modulated in darkness to allow for adequate electron flow to occur between the newly synthesized PSII and PSI units.

Young etiolated bean leaves exposed to CL[2] accumulate Chl *a* and Chl *b* without lag-phase (3), and they form complete (large in size) photosynthetic units. The concentration of the PSII and PSI units per chloroplast increases with the time of exposure to CL, until it reaches the concentration of the mature chloroplast.

Under certain conditions, but always in cases where the thylakoid is still in the process of development, the thylakoid components seem to undergo a process of reorganization (2, 4). Such a case has been noticed recently in the PSII unit components of etiolated bean leaves exposed to CL for a short time and then transferred to darkness (11): after transfer to darkness Chl accumulation stops completely; however, the growth of the leaf continues, and thus the Chl per g fresh weight decreases. The decrease is larger in Chl *b* than in Chl *a*. If, however, the Chl content is expressed on a leaf basis, then one finds that the Chl *a* content remains constant, while that of Chl *b* declines drastically. The Chl *b* decrease is more pronounced in leaves transferred to darkness after short preexposure to CL, while it becomes negligible in leaves transferred to darkness after long preexposure to CL. The decrease in Chl *b* content is accompanied by a drastic loss of the LHC-II and its 25 kD polypeptide, and by a concomitant increase in CPa (the complex originating in the core of the PSII unit) and its 42 and 47 kD polypeptides. This loss of the LHC-II components is followed by unstacking of preexisting grana and appearance of single long lamellae. Furthermore, the PSII activity per Chl and per leaf increases after transfer to darkness and high light intensities are required for saturation; the half-rise time of the fluorescence induction in the presence of DCMU also increases. Since all these occur without any new Chl synthesis, the results have been explained as indicating that during the dark period some Chl *a* belonging to LHC-II is liberated and reorganized into new PSII units (mainly the core of the units). This occurs only in thylakoids still in the process of development (short preexposure to CL), and not in those which have acquired the organization of the mature chloroplast (long preexposure to CL). This was explained as reflecting the capacity of the chloroplast to synthesize reaction center components in the dark, whenever their content is inadequate. If this is true, then one might expect to find a similar situation prevailing with the PSI units as well. We thus undertook this study trying to establish: (a) whether a reorganization of the PSI unit may also occur after transfer to darkness; (b) whether the reorganization of the PSII and PSI units occurs in a parallel way or independent of each other; (c) whether the formation of new PSII and PSI units in the D is also followed by the formation of new electron carriers.

Our results suggest that parallel to the PSII unit reorganization, the PSI unit components are also reorganized. Thus, the LHC-I is destroyed, and its Chl *a* is liberated and used for the formation of new, small in size, PSI units. Furthermore, the amount of Cyt *f* and Cyt *b* is increased in darkness, and the electron flow between the newly synthesized PSII and PSI units is established.

MATERIALS AND METHODS

Plant Material. Six-d etiolated bean leaves, *Phaseolus vulgaris* (var. red kidney), grown and handled as before (8) were exposed to CL for different periods of time and were then transferred to darkness.

Chl Determination. Chl was determined spectrophotometrically according to MacKinney (24); it was extracted exhaustively from leaves according to Argyroudi-Akoyunoglou and Akoyunoglou (8).

PSII, PSI, and P700 Determination. Chloroplasts were isolated

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[2] Abbreviations: CL, continuous light; LHC-II, light-harvesting complex of PSII; CPa, chlorophyll protein *a*; LHC-I, light-harvesting complex of PSI; DCIP, dichlorophenolindophenol; DPC, 1,5-diphenyl-carbazide; MV, methyl viologen; CPIa, chlorophyll protein I*a*; CPI, chlorophyll *a*-rich P700-protein; LHC, light-harvesting complexes (LHC-II + LHC-I); D, dark.

as previously described (11); chloroplasts used for P700 determination had no BSA in the homogenization buffer. PSII activity was determined in chloroplasts after washing in Tris (30), as the rate of DCIP reduction in the presence of DPC (29). PSI activity was monitored by the rate of MV-mediated O_2 uptake in DCMU poisoned chloroplast suspension in a mixture containing DCIP and Na ascorbate (6, 19). For P700 determination the thylakoids were washed once with 0.05 M Tricine-NaOH (pH 7.3), and were resuspended in the same buffer, containing 0.5% Triton X-100 (25), to give a concentration of about 20 μg/ml. P700 was determined from the ferricyanide-oxidized minus ascorbate-reduced difference spectrum (26), using a mM extinction coefficient of 64 (21). A Perkin-Elmer Double Beam 356 spectrophotometer was used.

Electrophoretic Analyses. Solubilization of thylakoids and electrophoretic analysis of pigment-protein complexes was done as in Argyroudi-Akoyunoglou and Akoyunoglou (10). Electrophoretic profiles were obtained in a Joyce-Loeble Chromoscan, and the distribution of Chl among the complexes was estimated on the basis of the area under each peak, by weight. Solubilization of thylakoids and electrophoretic analysis of thylakoid polypeptides was done as in Hoober *et al.* (22). Labeled polypeptides in polyacrylamide gel were detected by fluorography according to Bonner and Laskey (16).

Fluorescence Measurements. Low temperature fluorescence spectra of total leaves were measured with a set up described earlier (9), by immersing the samples into a glass Dewar flask, made to fit the cuvette compartment, filled with liquid N_2.

Determination of Cytochromes. Chloroplasts were isolated according to Rich and Bendall (27), and suspended in 50 mM Mes-NaOH (pH 6.0) (5), to give a concentration of about 22 μg Chl/ml. Cyt *f*, Cyt *b*-559$_{HP}$, and Cyt *b*-559$_{LP}$ were determined, as previously described (13), from the reduced minus oxidized difference spectra. Cyt *b*-563 was determined from the dithionite-reduced minus menadiol-reduced difference spectrum, 15 min after the addition of dithionite, to ensure complete reduction of the Cyt (27). A mM extinction coefficient of 22 was used for Cyt *f* and a mM extinction coefficient of 20 for Cyt *b* (13).

RESULTS

Table I shows the PSI activity, in comparison to the PSII activity, of chloroplasts isolated from 6-d etiolated bean leaves exposed to CL for various periods of time and then transferred to darkness. During the D period, parallel to the increase of the PSII activity an increase in the PSI activity also takes place. The increase, however, is lower in PSI than in PSII, and thus the PSII/PSI ratio, which is constant throughout the CL exposure, increases. This indicates that during the D incubation more PSII and PSI units are formed; moreover, it shows that the rate of synthesis of the PSII units is much higher than the rate of synthesis of the PSI units. For example, in leaves exposed to CL for 24 h and then transferred to darkness for 48 h the concentration of the PSII units (PSII/leaf) increases 4.2 times, and it reaches the final concentration of the green leaves (65 h CL), while the concentration of the PSI units increases only 1.8 times, and it remains about 3 times lower than the final concentration of the green leaves. In other words, if we assume that for mature bean chloroplasts the PSII/PSI ratio is equal to unity, then the PSII/PSI ratio increases from the value of 1.1 in 24-h CL to 2.6 after 48 h in darkness.

That new PSI units are formed is also evident from the P700 content of the plastids under these conditions. The P700/leaf ratio increases after transfer to darkness, and its increase follows closely the increase of the PSI activity (Table II). Thus, the PSI/P700 ratio has a constant value in all states studied (Table I).

All these changes are more pronounced when the leaves are transferred to darkness after a short preexposure to CL. As the time of preillumination increases the changes become smaller, and finally, in leaves transferred to darkness after 65 h in CL, both PSII and PSI activities, and the P700 content stop from changing.

Since the changes described occur with no net Chl synthesis per leaf, as shown in Table II, the results indicate that some of the Chl *a* in the dark transferred leaves is organized into new PSI units. These new PSI units seem to be of smaller size than those present in the leaves prior to transfer to darkness, since higher light intensity is required for saturation of their photosynthetic activity (Fig. 1).

To check further the PSI unit reorganization in the D, we estimated the pigment distribution among the pigment-protein complexes. As it has been previously described (11), in leaves transferred to darkness after a short preexposure to CL (24 h CL), the concentration of the LHC-II declines, while the concentration of the CPa is greatly increased. Thus, the LHC-II/CPa

Table I. *PSII, PSI Activities, and PSI/P700, PSII/Cyt f, PSI/Cyt f, PSII/PSI Ratios of Chloroplasts Obtained from 6-d Etiolated Bean Leaves Exposed First to CL and then Transferred to Darkness (D)*

Sample	Activity (V_{max})[a]				PSI/P700	PSII/Cyt *f*	PSI/Cyt *f*	PSII/PSI
	PSII[b]	PSI[c]	PSII	PSI				
	μmol/mg Chl·h		*μmol/leaf·h*			*ratios*[d]		
14 h Cl	513	218	9.8	4.2	1.1	1.0	0.9	1.1
14 h CL + 24 h D	1667	356	30.3	6.5	1.1	1.0	0.4	2.3
14 h CL + 48 h D	2857	526	49.1	9.0	1.1	1.0	0.3	2.7
24 h CL	500	213	21.9	9.4	1.1	1.1	1.0	1.1
24 h CL + 24 h D	1428	323	54.5	12.3	1.0	1.1	0.5	2.2
24 h CL + 48 h D	2439	455	92.4	17.2	1.1	1.1	0.4	2.6
40 h CL	500	213	44.6	19.0	1.0	1.1	1.0	1.1
40 h CL + 24 h D	833	267	67.6	21.6	1.0	1.1	0.7	1.5
40 h CL + 48 h D	1333	357	100.1	26.8	1.1	1.0	0.6	1.8
65 h CL	400	197	98.0	48.3	1.0	1.0	1.0	1.0
65 h CL + 24 h D	402	195	98.9	48.0	1.0	1.0	1.0	1.0
65 h CL + 48 h D	395	202	96.7	49.4	1.0	0.9	1.0	1.0

[a] V_{max} was determined from the intercept of the plot 1/V *versus* 1/I at 1/I = 0 (V is the rate of the reaction and I the incident light intensity.) [b] PSII activity, μmol DCIP reduced. [c] PSI activity, μmol O_2 consumed. [d] PSI/P700, PSI/Cyt *f*, PSII/Cyt *f* and PSII/PSI ratios were estimated on the assumption that in green leaves (65 h CL) they are equal to 1.

Table II. *P700 and Cytochrome Content of Chloroplasts Obtained from 6-d Etiolated Bean Leaves Exposed First to CL and then Transferred to Darkness (D)*

Sample	Chl *a*	Chl *b*	P700	Cyt *f*	Cyt *b*-555$_{HP}$	Cyt *b*-559$_{LP}$	Cyt *b*-563
	μg/leaf				*nmol/leaf*		
14 h CL	14.4	4.8	0.05	0.04	0.03	0.10	0.11
14 h CL + 24 h D	15.4	2.8	0.08	0.13	0.06	0.32	0.34
14 h CL + 48 h D	16.1	1.1	0.11	0.22	0.08	0.54	0.56
24 h CL	31.7	12.2	0.11	0.08	0.08	0.19	0.21
24 h CL + 24 h D	30.6	7.6	0.16	0.21	0.14	0.47	0.54
24 h CL + 48 h D	32.4	5.5	0.20	0.35	0.18	0.76	0.83
40 h CL	63.7	25.5	0.24	0.17	0.21	0.40	0.38
40 h CL + 24 h D	63.4	17.7	0.28	0.26	0.29	0.62	0.60
40 h CL + 48 hD	62.6	12.5	0.33	0.42	0.33	0.97	0.98
65 h CL	172.9	72.1	0.64	0.42	0.69	1.03	1.10
65 h CL + 24 h D	173.7	72.4	0.66	0.42	0.66	1.08	1.13
65 h CL + 48 h D	172.7	72.0	0.64	0.44	0.71	1.10	1.17

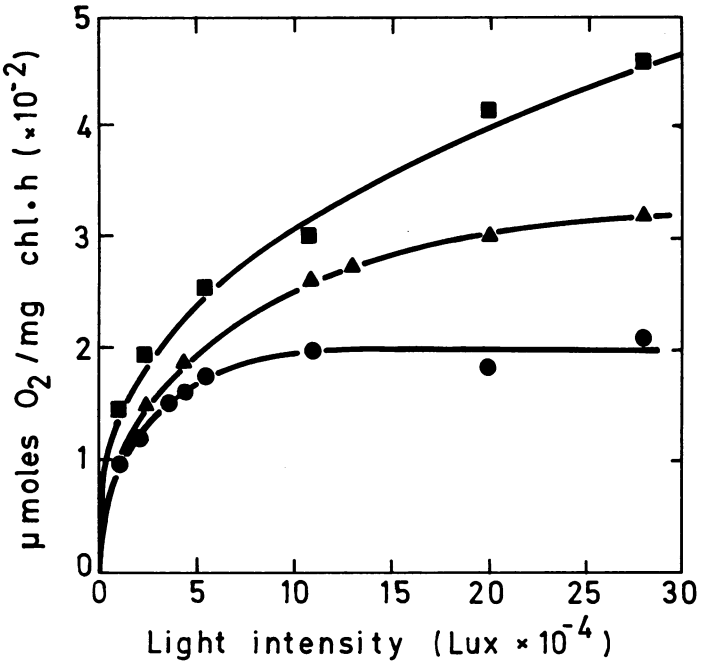

FIG. 1. Light saturation curves for PSI activity of chloroplasts obtained from 6-d etiolated bean leaves exposed first to CL for 14 h (●) and then transferred to darkness for 24 h (▲) or 48 h (■).

ratio drops from a value of 17 (24 h CL) to about 2 (24 h CL + 48 h D). Parallel to these changes, a decrease in the concentration of the CPIa and an increase in the concentration of the CPI is also observed in the dark. Thus, the CPIa/CPI ratio drops from a value of 0.6 (24 h CL) to 0.25 (24 h CL + 48 h D). The CPIa is a highly organized complex derived from the PSI unit, containing the core complex CPI, and the light-harvesting antenna complex of PSI, LHC-I (7, 23). The CPIa can be easily dissociated into its components, CPI and LHC-I (7, 23). The decrease, therefore, in the concentration of the CPIa during the dark period may be due to the decrease of the LHC-I.

This is clearly reflected in the polypeptide composition shown in Figure 2. In each pair of samples (*e.g.* 14 h CL and 14 h CL + 48 h D), the same amount of Chl *a* was loaded on the gel. Since the Chl *a* content remains constant during the dark period (Table II), the thylakoid polypeptide pattern shows the real changes in the concentration of the polypeptides in each case. As it is evident, parallel to the decrease in the 25 kD polypeptide, component of LHC-II, a decrease in the 24, 23, and 21 kD polypeptides, components of LHC-I (7, 23), is also observed in the D; on the other hand, the 47 and 42 kD polypeptides, components of CPa, and the 69 kD polypeptide, component of CPI, are increased. In addition to the above mentioned polypeptides, we noticed an increase in the 56, 53, 49, 32, 30, 29, 23.2, 21.5, 19.2, 18, 17.2, 16, 12.7, and 12 kD polypeptides, and a decrease in the 27 kD polypeptide during the dark period.

These changes are again less pronounced as the time of preexposure to CL is prolonged; finally, in thylakoids of leaves exposed to 65 h CL and then transferred to darkness, the Chl distribution among the pigment-protein complexes and the polypeptide composition remain unaltered (Fig. 2, slots 7 and 8).

That new polypeptides are synthesized in darkness and inserted into the developing thylakoid is further supported from *in vivo* labeling experiments, in which young etiolated bean leaves were exposed to CL for various periods of time and then transferred to darkness in the presence of [^{14}C]leucine. [^{14}C]Leucine was administered to the leaves by brushing 3 ml solution (0.08 μCi, 342 μCi/μmol) to 60 leaves; the leaves were washed with water 2 h prior to the isolation of the chloroplasts. The isolated chloroplasts were washed five times with Tricine to remove soluble proteins and any free [^{14}C]leucine that might be present, and then the ^{14}C-content of the thylakoids was determined. It was found that thylakoids incorporated [^{14}C]leucine (606,000 cpm/mg protein) in leaves exposed to CL for 14 h and then transferred to darkness for 24 h; on the contrary, in leaves exposed to CL for 65 h and then transferred to darkness for 24 h there was almost no incorporation of [^{14}C]leucine into the thylakoids (31,000 cpm/mg protein). To eliminate the possibility that the absence of ^{14}C-radioactivity in the thylakoids of the 65-h CL sample is due to the inability of the [^{14}C]leucine to penetrate the leaves, the Tricine washings were pooled together, centrifuged at 240,000*g* for 30 min to remove any insoluble material, and then the soluble proteins present in the supernatant were precipitated by TCA. The precipitate was washed, dissolved in 0.1 N NaOH containing 2% Na_2CO_3, and the ^{14}C-radioactivity and protein content was determined. It was found that the proteins were ^{14}C-labeled (145,000 cpm/mg protein), indicating that the [^{14}C] leucine penetrates into the leaves, and moreover, that during the D period a number of the soluble chloroplast proteins continue to be synthesized.

To check which thylakoid polypeptides were ^{14}C-labeled, and moreover, to eliminate the possibility that the ^{14}C-radioactivity of the thylakoids is due to contamination, the thylakoid samples were further analyzed by SDS-PAGE and the ^{14}C-labeled poly-

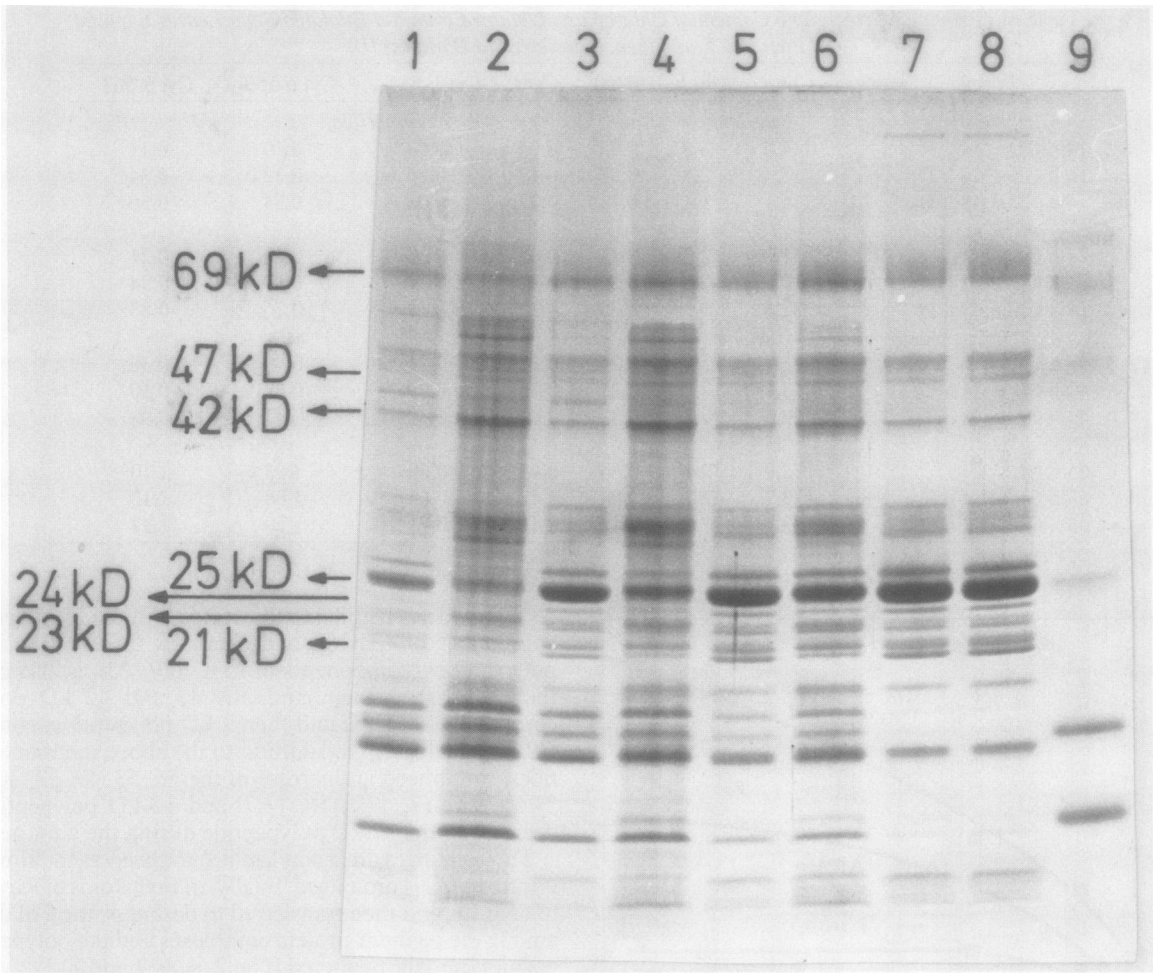

FIG. 2. SDS-(12.5-17%) PAGE of thylakoid polypeptides obtained from 6-d etiolated bean leaves exposed first to CL for 14 h (slot 1), 24 h (slot 3), 40 h (slot 5) or 65 h (slot 7), and then transferred to darkness for 48 h (slots 2, 4, 6, and 8, respectively). 4.5 μg Chl *a* was loaded on slots 1 and 2, 7 μg Chl *a* on slots 3 and 4, 9.5 μg Chl *a* on slots 5 and 6, and 10.5 μg Chl *a* on slots 7 and 8. Slot 9, standard proteins (ovotransferrin, 76 kD; BSA, 67 kD; ovalbumin, 45 kD; chymotrypsinogen A, 25.7 kD; myoglobin, 17.2 kD; Cyt *c*, 12.3 kD). The 21 kD polypeptide, under different gel or sample conditions, runs as two distinct polypeptides (7, 23).

peptides were detected by fluorography (Fig. 3). It is obvious that in the case of the 14-h CL + 24-h D sample the 69, 47, and 42 kD polypeptides, and a great number of others, are ^{14}C-labeled; in the case of the 65-h CL + 24-h D sample none of them are ^{14}C-labeled. In addition the fluorogram shows that the 25, 24, 23, and 21 kD polypeptides are also ^{14}C-labeled, indicating that the LHC-II and LHC-I apoproteins continue to be synthesized during the D period. This is in agreement with the results of Bennett (14) who found that, after initiation by 24 h of illumination, the synthesis of the LHC-II apoprotein continues in darkness at a readily detectable rate. Our results show that not only the LHC-II apoprotein, but also the LHC-I apoproteins continue to be synthesized in darkness.

The decrease in the concentration of the LHC-I in the dark is also supported from fluorescence measurements. It has been shown (12) that there is a good correlation between the changes in the 77°K fluorescence emission spectra of developing plastids and the step-wise synthesis, assembly and organization of the pigment-protein complexes; moreover, that the detection of the 730 to 735 nm band depends on the synthesis of LHC-I and its incorporation along with CPI into CPIa (7, 12, 23). For example, the intermittent light leaves contain chloroplasts which have only the core complexes of the PSII and PSI units (CPa and CPI, respectively). The 77°K emission spectra of these leaves show the 690 nm peak; the maximum at 735 nm (due to the antennae of PSI) is not present, but a small peak is noticed at 725 nm, which is probably due to the core of the PSI unit. After transfer of the intermittent light leaves to CL the monomer of LHC-II and the LHC-I are synthesized. Gradually, the LHC-II gives rise to its oligomeric forms, and the LHC-I is incorporated along with CPI into CPIa. Concomitantly, the 725-nm peak is gradually red-shifted reaching the wavelength of 735 nm, and also increases reaching values higher than the value at 690 nm; at the same time the maximum at 690 nm is also red-shifted reaching the wavelength of 696 nm. Experiments with isolated chloroplasts where the 77°K emission spectra were taken in the presence of the internal standard phycocyanin have shown that upon transfer of the intermittent light leaves to CL both F_{690} and F_{730} emission peaks increase. The increase, however, is greater at 730 nm than at 690 nm, so that as greening proceeds the F_{730}/F_{690} ratio continuously increases, and finally reaches the value of the green leaves chloroplasts (12). Accordingly, one should expect a high 730-nm emission band in the spectra of leaves exposed to CL for 14 h, where all the complexes are formed, and a low emission at 730 nm in the same leaves after being transferred to darkness, where dissociation of some of the CPIa and destruction of LHC-I takes place (Fig. 4). The 730-nm emission band of the leaves greened in CL for 14 h is high compared to the 690-nm band

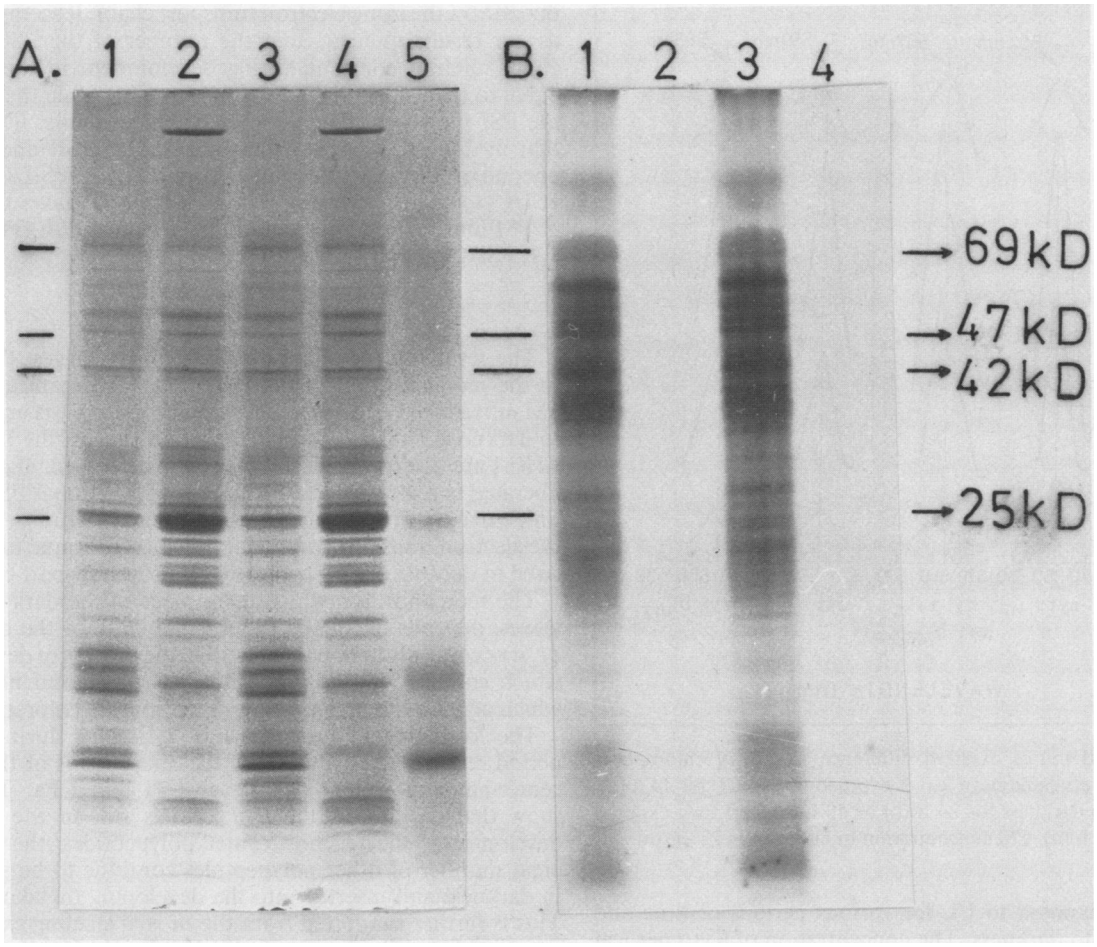

FIG. 3. A, SDS-(12.5–17%) polyacrylamide slab gel electrophoresis of thylakoid polypeptides obtained from 6-d etiolated bean leaves exposed first to CL for 14 h (slots 1 and 3) or 65 h (slots 2 and 4) followed by transfer to darkness for 24 h in the presence of ^{14}Cleucine; 100 μg protein was loaded on slots 1 and 2, and 120 μg protein on slots 3 and 4. Slot 5, standard proteins as in Figure 2. B, Fluorogram of the same slab gel.

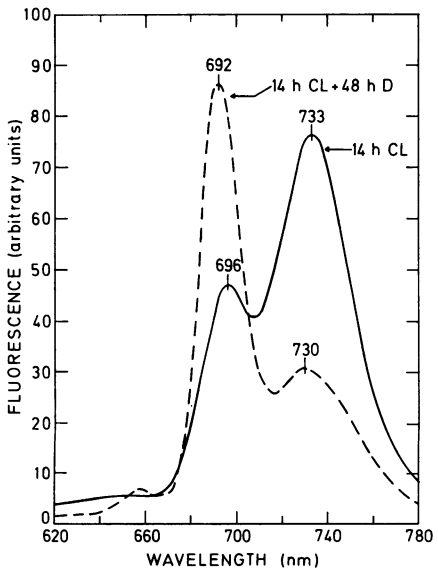

FIG. 4. Low temperature fluorescence spectra of 6-d etiolated bean leaves exposed first to CL for 14 h (——) and then transferred to darkness (D) for 48 h (– – –).

(F_{730}/F_{690} = 1.71), reflecting the synthesis of LHC-I and its association with CPI into CPIa. Upon transfer to darkness the F_{730}/F_{690} ratio declines gradually; for example, it is 1.70 after 20 min in darkness, 1.57 after 75 min, 1.10 after 5 h, 0.76 after 24 h, and 0.35 after 48 h in darkness. At the same time the maxima at 696 nm and 733 nm are blue-shifted reaching the wavelength of 692 nm and 730 nm, respectively, after 48 h in darkness. The changes observed in the 77°K emission spectra of the leaves after transferring them from CL to darkness are reversed to those taking place during greening, *i.e.* after transferring the intermittent light leaves to CL described above, and indicate the gradual dissociation of CPIa and the destruction of LHC-I. The fact that the long wavelength peak does not reach the 725 nm wavelength observed in the intermittent light leaves, shows that part of the CPIa remains still undissociated after 48 h in darkness.

To see whether the formation of the new PSI and PSII units in the dark is accompanied by formation of the electron transport chain components, we studied the formation of the thylakoid Cyt (Cyt *f*, Cyt *b*-559$_{HP}$, Cyt *b*-559$_{LP}$, Cyt *b*-563). Figure 5 shows the reduced minus the oxidized difference spectra of chloroplasts isolated from young etiolated bean leaves exposed to CL for 14 h (a), transferred to darkness for 24 h (b) or 48 h (c), and then exposed again to continuous light for 24 h (d). Since the amount of Chl is the same in all samples, it is clear that, during the dark period, the concentration of the Cyt is greatly increased. Table II shows the changes in the concentration of the thylakoid Cyt

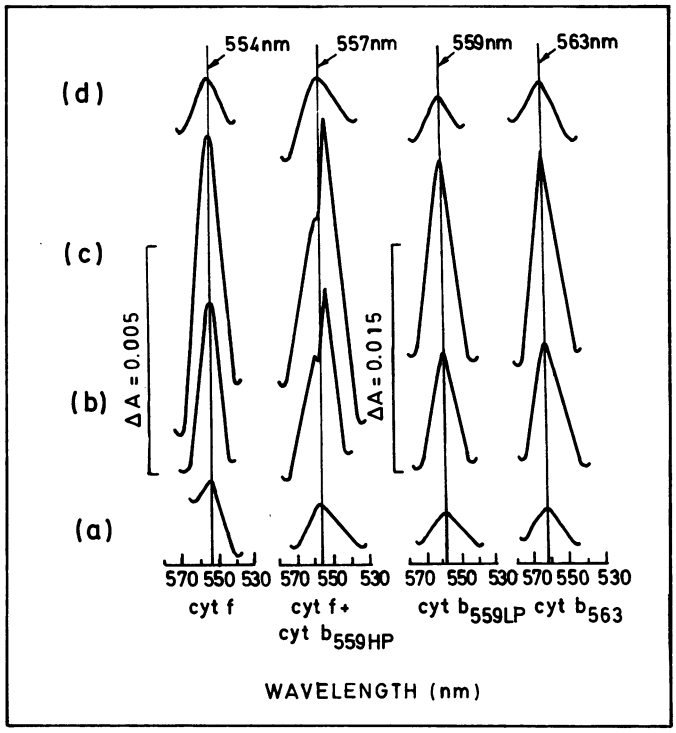

FIG. 5. Reduced minus oxidized difference spectra of chloroplasts obtained from 6-d etiolated bean leaves exposed first to CL for 14 h (a), then transferred to darkness for 24 h (b) or 48 h (c), and then exposed again to CL for 24 h (d). Chl concentration in all samples 22 μg/ml.

of chloroplasts exposed to CL for various periods of time and then transferred to darkness. The concentration of Cyt *f* per leaf increases 5.5 times in leaves transferred to darkness for 48 h after preexposure to CL for 14 h. The increase becomes smaller as the preexposure time to CL increases, and finally stops.

The increase in the Cyt *b*-559$_{LP}$ and Cyt *b*-563 content, in the dark transferred leaves, follows closely in all cases the increase in the Cyt *f*, while the increase in the Cyt *b*-559$_{HP}$ is less pronounced; *e.g.* in leaves transferred to darkness for 48 h after being exposed to CL for 14 h the concentration of Cyt *f*, Cyt *b*-559$_{LP}$, and Cyt *b*-563 per leaf increases 5 to 5.5 times, while the concentration of the Cyt *b*-559$_{HP}$ increases about 2.5 times (Table II). Various hypotheses have been proposed on the role of Cyt *b*-559$_{HP}$ in photosynthesis. According to Henningsen and Boardman (20), Cyt *b*-559$_{HP}$ is not essential for O_2 evolution, and it accumulates parallel to the formation of the LHC-II complex. Similarly, according to Gregory and Bradbeer (18), the Cyt *b*-559$_{HP}$ is located mainly in grana partitions, and its appearance in greening etiolated bean leaves coincides with the appearance of grana. On the contrary, according to Butler (17), Cyt *b*-559$_{HP}$ is involved in O_2 evolution. He suggested that a close physical association exists between the Cyt *b*-559$_{HP}$ and the O_2 evolving apparatus. Both hypotheses can explain our results since, during the dark period, the LHC-II complex declines drastically and unstacking of some of the preexisting grana is observed, but also the newly synthesized PSII units have no active water-splitting capacity (11).

Considering the changes in the concentration of Cyt (*e.g.* Cyt *f*/leaf) and those of PSII and PSI units (PS activity/leaf) (Table I), we can conclude that, in leaves transferred to darkness after a short preexposure to CL (14 h or 24 h CL), the rate of Cyt formation (*i.e.* electron transport chain/leaf) follows closely the rate of PSII unit formation (PSII/Cyt *f* = constant), while the rate of PSI unit formation is much lower (PSI/Cyt *f* decreases). It has been proposed (15), that a number of PSII and PSI units operate on the same electron transport chain. It seems, therefore, as our results indicate, that the number of the PSII units per chain remains constant during development in the dark, and equal to that of the green leaves (65 h CL), while the number of the PSI units per chain is smaller. For example, if we assume that in 24 h CL leaves equal numbers of PSII and PSI units operate on the same electron transport chain (PSII/Cyt *f* = 1.1, PSI/Cyt *f* = 1.0), then in 24 h CL + 48 h D leaves the number of PSI units per chain is two-fifths of that of PSII (PSII/Cyt *f* = 1.1, PSI/Cyt *f* = 0.4) (Fig. 6).

DISCUSSION

The results of the present study strongly suggest that parallel to the reorganization of the PSII unit, a reorganization of the PSI unit also takes place in developing chloroplasts upon transfer to darkness. During this reorganization process the LHC-II and LHC-I are disorganized, their Chl *b* is destroyed, and the Chl *a* liberated is reused for the formation of additional PSII and PSI units, mainly the core of the units. These new units, along with the electron transport components, which continue to be synthesized in darkness, form complete electron transport chains.

The reorganization which takes place in the dark transferred leaves, depends on the developmental stage of the chloroplast, *i.e.* it occurs only in thylakoids still in the process of development, which contain reduced amount of PS units, and not in those which acquire the organization of the mature chloroplast.

The formation of new PSI and PSII units during the dark period requires the synthesis of the apoproteins of the reaction center-antenna-protein complexes CPI and CPa. The results show that as long as the thylakoid is still in the process of development, the reaction center polypeptides, the Cyt and a great number of other polypeptides continue to be synthesized in darkness and inserted into the developing thylakoid (Fig. 2). This is further supported from the *in vivo* labeling experiments, which additionally show that the LHC-I and LHC-II apoproteins are also synthesized in darkness, even though they do not accumulate. It seems, therefore, that in darkness a great number of the thylakoid polypeptides are synthesized; the LHC-apoproteins, however, not being able to be stabilized in the absence of Chl synthesis, are digested.

Since no Chl *a* is formed during the dark period, the newly synthesized reaction center polypeptides use the Chl *a* already bound on the light-harvesting complexes, LHC-I and LHC-II, to form the new PSI and PSII units. The question that arises is whether, after transferring the plants from light to darkness, (a) the apoproteins of the PSI and PSII reaction centers are first synthesized, inserted into the thylakoid, compete for the Chl *a* already bound to LHC-I and LHC-II, remove it to form CPI and CPa, and then the LHC-I and LHC-II are disorganized and their apoproteins are digested; or (b) the LHC-I and LHC-II are first disorganized, and then their Chl *a* becomes available to bind to the apoproteins of the PSI and PSII reaction centers. In the first

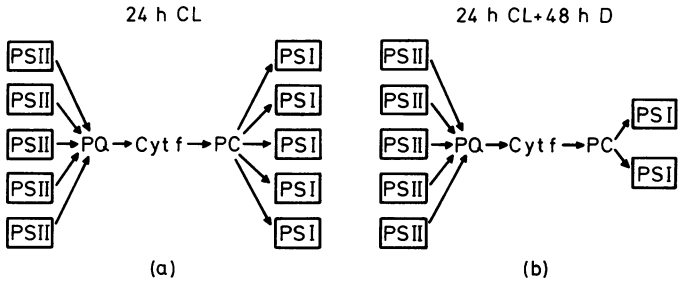

FIG. 6. Schematic representation of the relative number of PSII and PSI units cooperating on the same electron transport chain of 6-d etiolated bean leaves exposed first to CL for 24 h (a) and then transferred to darkness (D) for 48 h (b).

case the destruction of the LHC-I and LHC-II would depend on the concentration of the PSI and PSII units present in the thylakoid, *i.e.* on the rate of synthesis of the reaction center polypeptides in the dark, which in turn would depend on preexposure time to CL. On the contrary, in the second case the destruction of the LHC-I and LHC-II would occur independent of the synthesis of the PSI and PSII reaction center apoproteins in the dark. We have tried to give an answer to this question earlier (1) by exposing etiolated bean leaves first to intermittent light for various times; in this case only small PSI and PSII units are synthesized, containing the core of the unit. The number of the units increased as preexposure time to intermittent light increased. These leaves were then transferred for a short time to CL, where accumulation of LHC-I and LHC-II took place, and the photosynthetic units increased in size. Thereafter, the leaves were transferred to darkness, and the effect of preillumination time in intermittent light on thylakoid reorganization was studied. It was found that the reorganization depends on the ability of the chloroplast to increase the number of the PS units, and reach the number found in mature chloroplasts, and not on the instability of the LHC-II, as it was suggested by Bennett (14).

It is not known whether the Chl *a* of LHC-II is used specifically for the formation of the PSII units, and the Chl *a* of LHC-I for the PSI units, or the Chl *a* of both LHC is used unspecifically for the formation of both PS units. The first possibility seems to be more probable, since, as it has been previously shown (28), the growth of the PSII unit is independent of that of the PSI unit. Furthermore, it was found that, when young etiolated bean leaves exposed to intermittent light (8) for long periods of time are transferred to CL in the presence of the protein synthesis inhibitor chloramphenicol, then a reorganization of the PSII unit components takes place, and Chl *a* of CPa is used for the formation of LHC-II, but not for the formation of LHC-I (4).

The drastic increase in the PSII/PSI ratio taking place during the dark incubation indicates that the rate of formation of the PSI and PSII units is different in darkness than in light; and that darkness affects especially the rate of formation of the PSI units. Similar effects were also observed in bean leaves greened under intermittent light; the PSII/PSI ratio in these plastids is 2.5 times higher than that of the mature chloroplast. It is difficult to say whether darkness affects the rate of synthesis of the reaction center apoproteins, which has an effect on the rate of formation of the PSI and PSII units, or if there is a different kind of control.

A continuous reorganization of the thylakoid components during development seems to take place also in nature. Preliminary experiments in our laboratory have shown that when young etiolated bean leaves are exposed to the day-night regime, the Chl *a*/Chl *b* ratio changes during the night. It was found, for example, that in the first d of greening the Chl *a*/Chl *b* ratio was low at the end of the d and high in the beginning of the next d. The change in the Chl *a*/Chl *b* ratio during the night continued up to 4 d and then stopped.

The disorganization of the pigment-protein complexes, and the reassembly of Chl *a* into new and different pigment-protein complexes during chloroplast development, seems to be a general mechanism in thylakoid biosynthesis. Moreover, the digestion of the newly inserted, but still unstable polypeptides, and those which become unstable during the reorganization process, may be one of the mechanisms which control the growth and differentiation of the photosynthetic membrane (see Akoyunoglou and Akoyunoglou [1]). It is not known whether this control mechanism operates in all biological membranes. This possibility cannot be excluded.

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