FORMATION OF TWO CHLOROPHYLL-PROTEIN COMPLEXES DURING GREENING OF ETIOLATED BEAN LEAVES

J.H. Argyroudi-Akoyunoglou, Z. Feleki and G. Akoyunoglou,
Department of Biology, Nuclear Research Center "Democritus",
Athens, Greece.

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Summary. Etiolated bean leaves exposed to a series of light-dark cycles are devoid of Chl b[1] and contain agranal chloroplasts. The lamellae of these chloroplasts are deficient in the chlorophyll-protein complex II, propably associated with PS II in vivo.

During our studies on the development of the photosynthetic apparatus we found that Chl a can be selectively synthesized in greening etiolated bean tissue under a system of light-dark cycles (2 min white light alternating with 98 min dark period) (1) so that very high ratios (50 to 300) of Chl a to Chl b are established. The possibility that the flashed leaves may form only the Chl a containing photosystem I (PS I) was examined by studying the distribution of the selectively formed Chl a between the solubilized by SDS two major chlorophyll-protein complexes of chloroplast lamellae, or between the subchloroplast particles obtained by digitonin disruption.

Etiolated bean plants Phaseolus vulgaris (red kidney variety) were harvested at 4 to 6 days of age. After the removal of one cotyledon, the leaves were placed on moist filter paper in covered petri dishes, and were exposed to the intermittent or continuous illumination (1). Seven grams of leaves were withdrawn immediate-

[1]Abbreviations: Chl: chlorophyll; SDS: sodium dodecyl sulfate; δ-ALA: δ-aminolevulinic acid; DCIP: dichlorophenolindophenol. PS I: photosystem I; PS II: photosystem II.

ly after the last flash of light and were ground at full speed for 30 sec in a Waring blendor with 25 ml sucrose buffer (0.5 M sucrose buffered at pH 7.4 with 0.1 M potassium phosphate and 0.01 M EDTA), in a cold room at 4°C. Following filtration through 4 layers of cheece cloth, the homogenate was centrifuged at 6,000 x g for 20 min, and the pellet obtained was suspended in 20 ml of water using a Sorvall Omni-mixer at full speed for 1 min. The chloroplasts were centrifuged at 36,000 x g for 30 min and the pellet was treated in a Potter-Elvejem homogenizer with 1 ml 1% SDS in 0.1 M phosphate buffer pH 7.0, or with 1 ml 0.5% SDS in 0.1 M Tris-HCl buffer pH 8.0. The suspension was centrifuged at 36,000 x g for 10 min and the chlorophyll-protein complexes of the solubilized lamellae were separated by hydroxylapatite column chromatography or gel electrophoresis. The molar ratio of SDS/Chl in the chloroplast extracts was between 50 and 100.

After this treatment all the lamellar material entered the

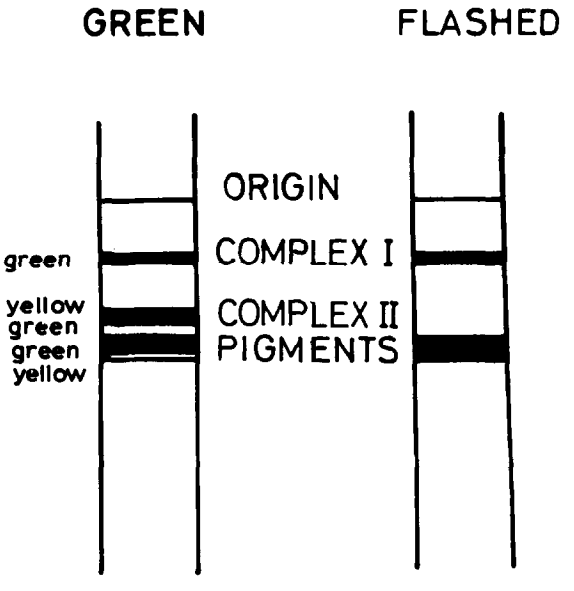

Figure 1. Gel electrophoresis of the solubilized by SDS chloroplast lamellar chlorophyll-protein complexes I and II of etiolated bean leaves exposed to 72 hours continuous light or 46 light-dark cycles.

gels on electrophoresis. The electrophoretic pattern of the extracts of green bean leaves was very similar to that obtained by Thornber et al (2). Two chlorophyll-protein complexes were present. Contrary to that, electrophoresis of the extracts of flashed bean leaves showed only one chlorophyll-protein band with an R_f equal to that of complex I of green tissue (Figure I). The electrophoretic pattern of the same bean plants, after their exposure to continuous illumination, showed clearly the two chlorophyll-protein bands of green plant lamellae. The formation of the two chlorophyll-protein complexes was further studied by hydroxylapatite column chromatography (3). Calcium phosphate was prepared according to Siegelman et al (4) and transformed to hydroxylapatite by titration with 2 N KOH at room temperature to a

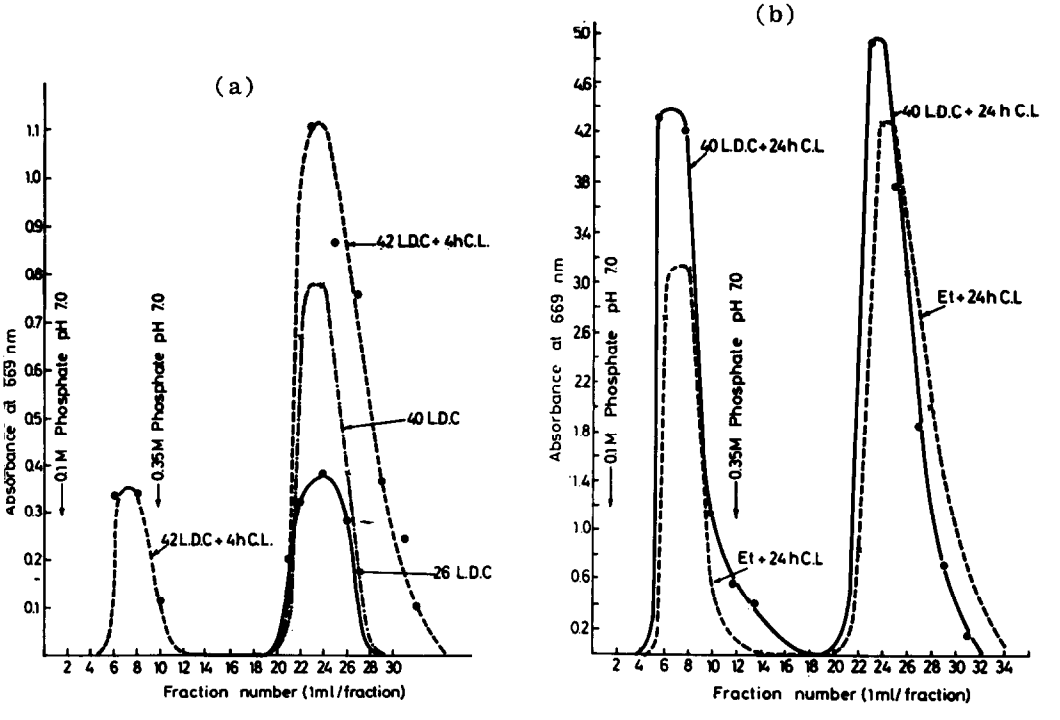

Figure 2. Hydroxylapatite column chromatography of the solubilized by SDS chloroplast lamellar chlorophyll-protein complexes of etiolated bean leaves exposed to light-dark cycles (LDC) or continuous light (CL).

pH of about 9.0. The columns (1 x 5.5 cm) were washed prior to use with 5 ml 1% SDS in 0.1 M phosphate buffer pH 7.0. Stepwise elution was performed with 0.1 M phosphate buffer pH 7.0 followed by 0.35 M phosphate buffer pH 7.0. The spectra of the fractions eluted were recorded upon collection. Representative results of such experiments are shown in Figure 2. The chromatographic pattern of the solubilized lamellae of green plants shows two chlorophyll-protein complexes, eluted with 0.1 M (complex II) and 0.35 M (complex I) phosphate buffer respectively. On the contrary the flashed bean leaves show only the complex I chlorophyll-protein peak. The chlorophyll-protein complex II is formed after transfer of the plants to continuous illumination.

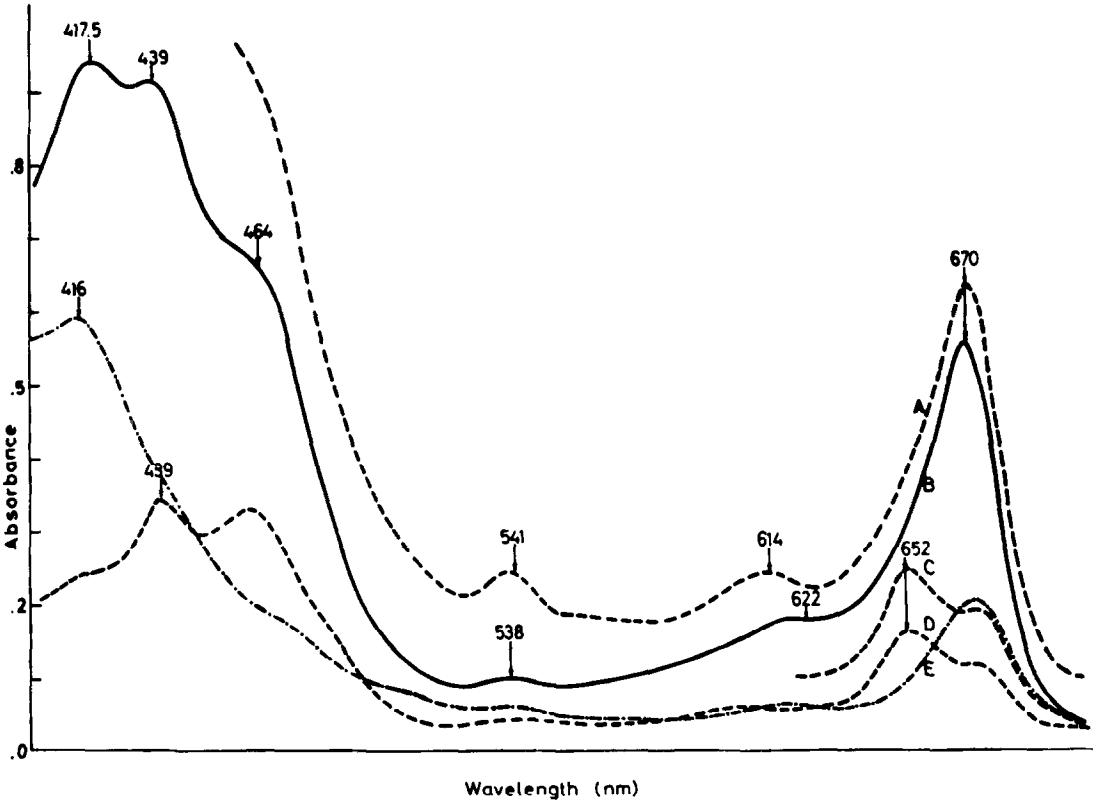

Figure 3. Absorption spectra of the chlorophyll-protein complexes eluted from hydroxylapatite columns with 0.35M (A,B,E) or 0.1M (C,D) phosphate buffer pH 7.0.
A: : tomato plants grown in a greenhouse.
B,C,D: etiolated bean leaves after 24 hrs continuous light.
E : etiolated bean leaves after 40 light-dark cycles.

The absorption spectra of the two chlorophyll-protein complexes are shown in Figure 3. The complex II chlorophyll-protein, eluted with 0.1 M phosphate buffer, contains Chl b and has absorption bands at 670 nm and 652 nm in the 650-700 nm region, while the complex I chlorophyll-protein eluted with 0.35 M phosphate buffer, contains predominantly Chl a and has a strong absorption band at 670 nm. Based on total absorption units applied on the columns, one can calculate approximate values for the amount of the chlorophyll-protein complexes of chloroplast lamellae formed during the greening process (Table I).

Table I. Distribution of chlorophyll between the solubilized by SDS lamellar chlorophyll-protein complexes in bean plants exposed to different illumination conditions.

Light Treatment	Chlorophyll-Protein Complexes (% Of total)	
	II	I
26 LDC	0	28
40 LDC	0	30
CL (24 hrs)	18	33
40 LDC+CL (4 hrs)	8	36
40 LDC+CL (24 hrs)	16	28

5 days old etiolated bean leaves were used after exposure to a number of light-dark cycles (LDC) or continuous illumination (CL). The values shown represent percentages of the total solubilized by SDS chloroplast lamellae, and are based on absorption at 669 nm.

Thornber et al (2) attribute the two chlorophyll-protein complexes to PS I and PS II. Unfortunately, the biochemical activities of these complexes can not be measured due to the SDS inactivation. We thus studied the distribution of chlorophyll between the subchloroplast particles enriched in each photosystem obtained by digitonin fragmentation (5). The chloroplast lamellae of bean leaves exposed to different light conditions were disrupted by 0.5% digitonin and the 10,000 x g (10 K) and 144,000 x g

(144 K) fractions (active in PS II and PS I reactions respectively (5)) were separated. The distribution of chlorophyll in these fractions is given in Table II. As it is shown 28% of the chlorophyll of leaves illuminated for 24 hours is in the 10 K fraction and 14% in the 144 K fraction. On the contrary, only 2% of the chlorophyll of flashed bean leaves is present in the 10 K fraction and 22% in the 144 K fraction. It is possible that the small amount of chlorophyll of the 10 K fraction is a contamination by the lighter components. The values of Anderson and Boardman (5) are 46% for the 10 K and 11% for the 144 K fraction, and are representative of mature spinach leaves. Indeed, when 10 days old etiolated bean leaves are exposed to continuous illumination for 24 hours, 47% and 10.3% of the chloroplast chlorophyll is found in the 10 K and 144 K respectively. This was expected, since Chl b is present in higher amount in the older plants (6), and the PS II, represented by the 10 K fraction, has also been found to be more abundant in the older plants (7).

Table II. Chlorophyll content of the 10 K and 144 K subchloroplast particles obtained by digitonin disruption of etiolated bean leaves exposed to continuous (C.L.) or intermittent (L.D.C.) illumination.

	5d+24 hrs C.L.		5d+29 L.D.C.		10d+24 hrs C.L.		Anderson and Boardman (5)	
Sample	Chl a / Chl b	Chl. %	Chl a / Chl b	Chl. %	Chl a / Chl b	Chl. %	Chl a / Chl b	Chl. %
Chloroplast Extract	2.2	100	8.5	100	2.24	100	2.83	100
10 K	1.8	28.5	5.1	2	1.7	47	2.27	46.2
144 K	2.9	14.3	19.3	22	4.25	10.3	5.34	11.7

Since in the flashed bean plants very little, if any, of chlorophyll is present in the 10 K (PS II) fraction, it seems propable that the missing chlorophyll-protein from these plants

is the one associated with the PS II complex of the lamellae. The selectively synthesized Chl a in the flashed bean leaves, however, is found mainly in the 144 K (PS I) fraction, and the chlorophyll-protein isolated after hydroxylapatite chromatography of the SDS solubilized lamellae may represent the chlorophyll-protein complex associated with PS I *in vivo*.

Electron micrographs of flashed bean leaves chloroplasts showed the presence of the "primary thylakoids" characteristic of the early stage of greening (11, 12). No indication of grana stacks was obtained; The latter were formed only after transfer of the plants to continuous illumination.

These findings coupled with the results on the formation of the two chlorophyll-protein complexes, suggest that the stroma lamellae structures may represent the Chl a-rich protein complex while the grana structures may represent the Chl b-rich protein complex, formed under continuous illumination. In this respect the recent work of Sane *et al.* (13) should be taken into account. The light fraction of the French-press separated photosystems, corresponding predominantly to PS I particles, seems to originate from stroma lamellae and the end membranes of grana stacks,while the heavy fraction, corresponding predominantly to PS II particles, originates from the partition region of grana stacks.

In addition, the chloroplasts of a barley mutant lacking Chl b had a lesser amount of chlorophyll in PS II, and when viewed under the electron microscope showed fewer grana and thylakoids per granum but more single lamellae (14, 15). Grana stacks, however, seem to be not essential for PS II, since this mutant exhibits high PS II activity. A mutant of *Chlamydomonas reinhardii* (ac-31) has also been found to have normal PS II activity, but essentially no stacking of lamellae (16). On the contrary the

results of Homann and Schmid indicate that grana are necessary for PS II activity in a number of Nicotiana tabacum mutants (17). Woo et al. (18) have found recently that the agranal chloroplasts of the bundle sheath cells of C_4-cycle plants are deficient in PS II units, and are characterized by an altered Chl a to Chl b ratio, a reduced Cyt b-559, and by the absence of PS II fluorescence signal. Bishop and coworkers (19), however, have found Hill activity in these chloroplasts, but $NADP^+$ could be reduced only in the presence of DCIP and ascorbate. Hence, they concluded that what is missing in these chloroplasts is not the PS II itself, but the link between the two photosystems. Recently, De Greef et al. (20) reported oxygen evolution from bean leaves which accumulated Chl a (but not Chl b) by a series of brief red light illuminations. This may suggest that a small fraction of the chlorophyll-protein complex of PS II in our system remains undetected by the analytical procedures we used.

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