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Organization of Chlorophyll *a* in the Light-Harvesting Chlorophyll *a/b* Protein Complex as Shown by Circular Dichroism[1]

LIQUID CRYSTAL-LIKE DOMAINS

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ABSTRACT

The development of thylakoid stacking, accumulation of the light-harvesting chlorophyll *a/b* protein complex (LHCP), and the changes of circular dichroism (CD) which reflect the organization of chlorophyll molecules in greening thylakoids of bean *Phaseolus vulgaris* cv Red Kidney leaves were investigated.

Chloroplasts formed under intermittent light contained large double sheets of membrane with extensive appression in addition to separate lamellae. Thylakoids of such chloroplasts were devoid of LHCP and exhibited a relatively small CD in the chlorophyll absorption region. Upon continuous illumination, the rearrangement of membranes to characteristic grana and the accumulation of the LHCP was accompanied by the gradual appearance of the very intense CD signal with peaks at 682 to 684 (+) and 665 to 672 nanometers (−). The magnitude of differential absorption was approximately 100 times larger than that of the chlorophyll *a* in solution. This suggests a superhelical liquid crystal-like organization for LHCP, a texture which can be altered by changes of the electric field in the photosynthetic membranes.

Chloroplasts exhibit a very intense CD[2], the origin and significance of which has not been completely elucidated (10, 14). The giant CD is connected only with granal chloroplasts (10). The extent of membrane stacking and the magnitude of CD cannot be well correlated; thus, the giant CD does not result from the stacking *per se* (8). It has been shown that the intense CD is not a measuring artefact (9, 14). In a work analyzing light-scattering effects on the CD of chloroplasts, attention has been called to the similarities between the CD enhancement observed in cholesteric liquid crystals and chloroplasts or algae (24). We have sought to identify the membrane constituent of granal chloroplasts responsible for the giant CD signal. In leaves greening under intermittent and continuous light, the appression of membrane pairs is somewhat separated in time from the synthesis of multilamellar grana containing LHCP. Our data suggest that the giant CD originates from LHCP which, in turn, means that LHCP must contain liquid crystal-like regions.

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[2] Abbreviations: CD, circular dichroism; LHCP, light-harvesting Chl *a/b* protein complex.

MATERIALS AND METHODS

Plant Material. Bean plants (*Phaseolus vulgaris* cv Red Kidney) were grown at 24 ± 1°C in complete darkness. After 4 d, the seedlings were illuminated by intermittent white light of 1000 lux intensity using 75-w tungsten lamps connected to an electronic timer programmed to provide an illumination in cycles of 2 min alternating with 98 min of darkness (2). After exposure to 48 light-dark cycles, the plants were further exposed for various periods of time to continuous light of 1500 lux at the level of the leaves.

From the leaf blades, 20- × 10-mm flat pieces were cut out, infiltrated with water to reduce light-scattering by the samples, transferred to 2-mm cuvettes, and used for spectropolarimetric measurements.

After the measurements, the pieces of leaves were extracted with acetone, the pigments were transferred to peroxide-free ethyl ether, and Chl content was determined spectrophotometrically by the two-wavelengths method (11).

Electron Microscopy. Small pieces of leaves were fixed in Karnovsky's medium (17) and Millonig's (20) osmic acid. The samples were dehydrated in ethyl alcohol and embedded in Durcupan (Fluka). Thin sections were cut on a Porter-Blum ultramicrotome and stained using Reynold's (25) procedure. Electron micrographs obtained with a JEOL 100/B electron microscope were analyzed for the lengths of linearly appressed or stacked membranes per unit area of sections. Results reported the characteristic values from three experiments.

Spectropolarimetry. CD measurements were made with JASCO 40C spectropolarimeter. The samples were placed close to the photomultiplier window (distance, 12 mm). The CD of greening chloroplasts was measured *in situ* (*i.e.* in the leaves). This procedure avoided the destruction of fragile chloroplasts by isolation and gave valid results since the CD spectra of green leaves and isolated green chloroplasts matched perfectly. The results reported were obtained from the same leaves used for measurements of membrane development.

RESULTS

Different Types of Thylakoid Appression between Primary and Granum Lamellae. In chloroplasts formed under intermittent light, a relatively abundant membrane formation occurred producing parallel thylakoids along the long axis of the plastid section. Some of these primary lamellae were arranged in pairs and were appressed to each other (Fig. 1a). In chloroplasts further illuminated with continuous light, granum formation started

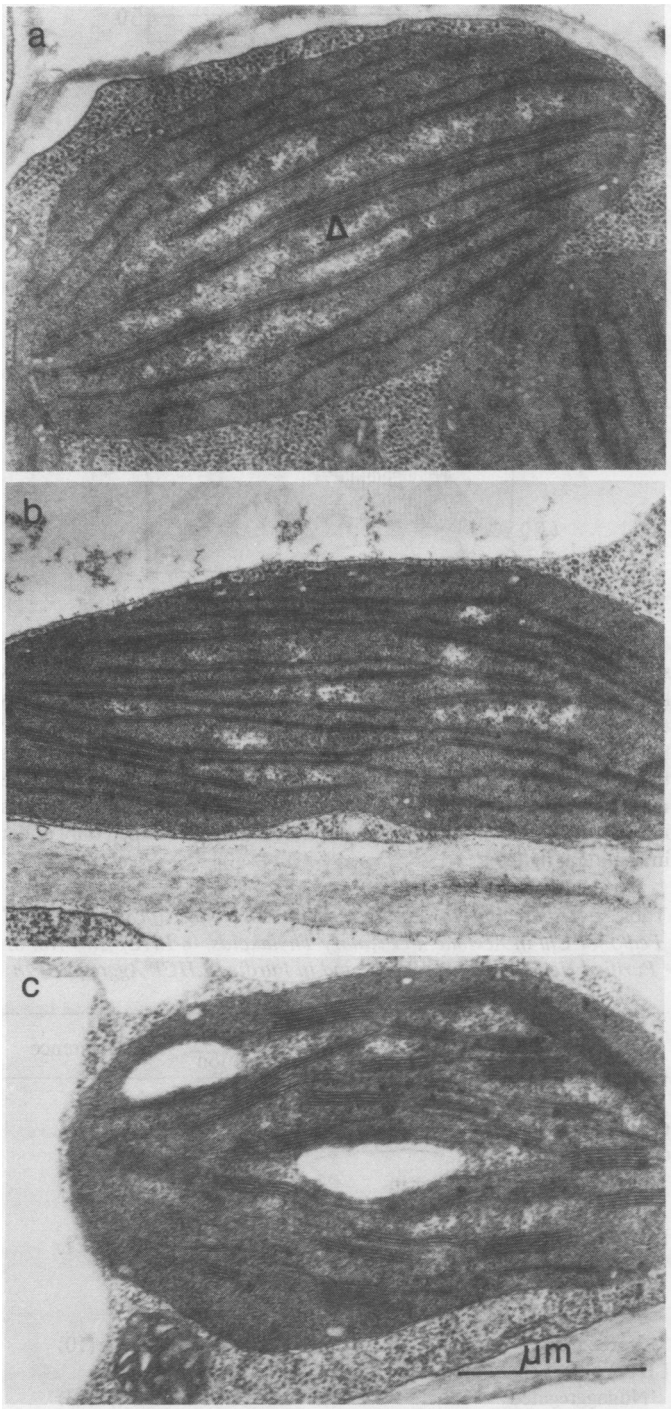

FIG. 1. Electron micrographs of chloroplasts from bean leaves grown under intermittent light and subsequent continuous illumination of various duration. a, 48 light-dark cycles; b, 48 light-dark cycles followed by 8 h of continuous light; c, 48 light-dark cycles followed by 24 h of continuous light. The arrow points to the large double sheaths characteristic of the membrane appression of such juvenile chloroplasts.

with a concomitant breakdown of the double lamellae (Fig. 1, b and c).

As a result of these two opposing processes, the proportion of stacked membranes did not increase appreciably (Fig. 2A). After 24 h of continuous illumination, practically all stacking was found in multilamellar grana, and long fusions connecting double lamellae disappeared (Fig. 2B). This structural rearrangement was accompanied by a rapid Chl *b* accumulation indicating the

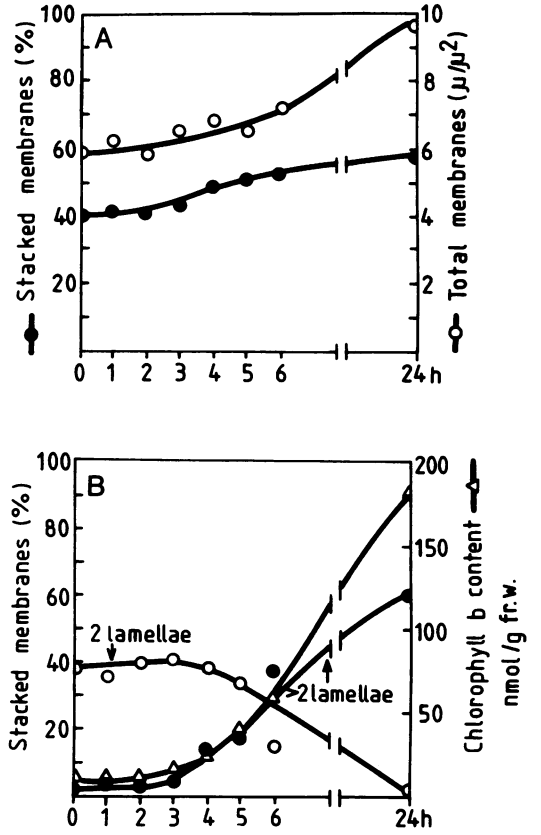

FIG. 2. Stacking characteristics of thylakoids after various periods of continuous illumination applied to bean leaves grown in intermittent light. 0 h corresponds to samples which obtained 48 light-dark cycles. A: (○), Total length of membranes; (●), percentage of appressed membranes. B: (○), Percentage of appressed membranes between double sheaths of lamellae; (●), percentage of appressed membranes in stacks of three lamellae or more; (△), Chl *b* content.

synthesis of LHCP.

Development of a Very Intense CD as a Function of LHCP Formation. Chloroplasts grown under intermittent light displayed a complex CD spectrum with bands at 665 nm (+), 675 nm (−), and 690 nm (−) and a trough at 682 nm (Fig. 3, 0^h). Changes in CD occurring during continuous illumination were characterized by the difference spectra calculated between samples illuminated with continuous light and those which were not. After a short lag-period, the CD of nonilluminated samples became gradually superimposed by a CD signal with components at 665 to 672 nm (−) and 680 to 684 nm (+) and a negative band at 650 nm (Fig. 3).

Inasmuch as the ratio of Chl *a* to Chl *b* in LHCP is approximately one, from the increment of Chl *b* content of the leaves one can estimate the amount of Chl *a* accumulated in LHCP and in the photosystems devoid of Chl *b*. This procedure revealed that LHCP formation starts shortly after the onset of continuous illumination, while photosystems do not incorporate new Chl for about 8 h. Development of the intense CD signal was fairly concomitant with the synthesis of LHCP and deviated from the accumulation of Chl in the photosystems (Fig. 4).

The differential absorption $A_L - A_R/A$ of LHCP in greening leaves was two orders of magnitude higher than that of the Chl in solution and one order of magnitude higher than the differential absorption of thylakoids synthesized under intermittent light. The differential absorption of nonaggregated LHCP separated from green leaves was similar to that of the latter, while

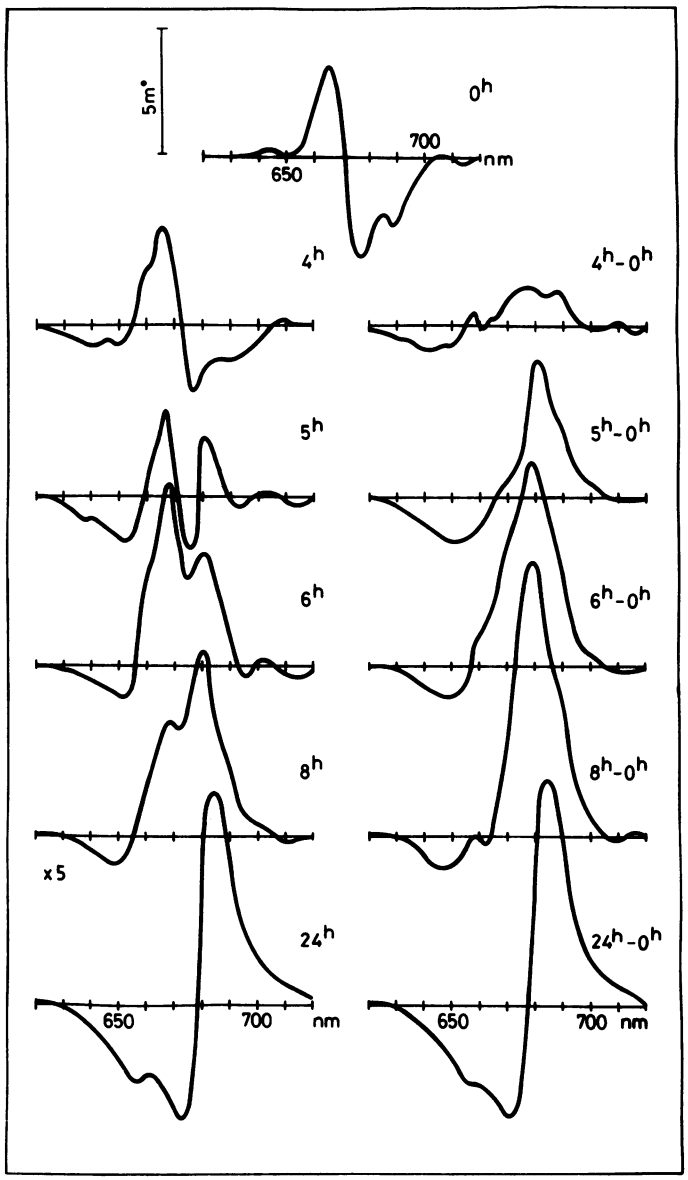

FIG. 3. CD spectra and changes of CD in bean leaves grown under intermittent light and subsequent continuous illumination. Top, CD spectrum of leaves obtained after 48 light-dark cycles; left side, spectra measured after various time periods of continuous illumination; right side, difference spectra calculated between samples illuminated with continuous light and those which were not.

the purified LHCP aggregated *in vitro* was practically the same as for LHCP in greening or green leaves (Table I).

DISCUSSION

In the protracted greening process induced by intermittent and continuous illumination, two stages of chloroplast development could be discerned: primary thylakoid formation and the granum formation. In the normal greening process, these two processes occur simultaneously (7). In both developmental stages, the extent of stacking was the same and both photosystems have been reported as being active (1). The most appreciable difference is that LHCP synthesis occurs only in the second stage, simultaneously with granum formation (3, 4). Thus, the comparison of chloroplasts in these two stages allows us to recognize the differences in thylakoid architecture and functional characteristics arising from the presence of LHCP.

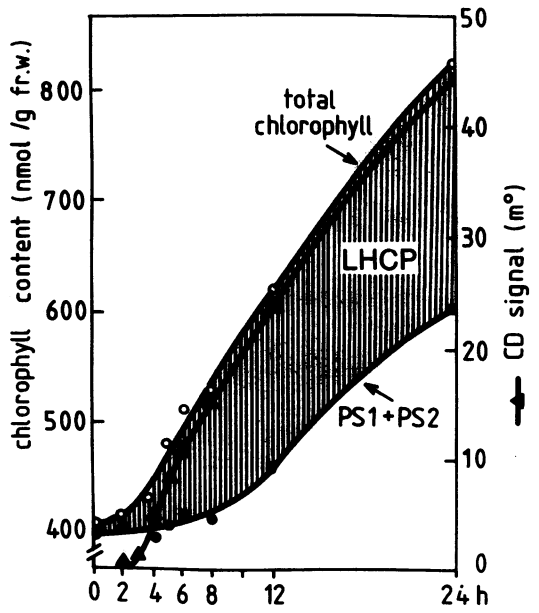

FIG. 4. Accumulation of LHCP and changes in the magnitude of CD signal during continuous illumination applied to bean leaves after 48 light-dark cycles. (O), Total Chl content; (●), total Chl content minus two times the Chl *b* content which corresponds to the Chl contained by the two photosystems. The difference (twice the Chl *b* content) represents the Chl incorporated into LHCP. (Δ), peak-to-peak magnitude of the main band in the difference spectra calculated between the CD of leaves illuminated with continuous light and those which developed only under intermittent light.

Table I. *Differential Absorption per Unit Absorbance (at the Main Red Peak) for Chl in Acetone, in Primary Thylakoids, in LHCP In Vivo, in Purified Nonaggregated LHCP, and in Purified LHCP Aggregated In Vitro*

Material	Differential Absorption	Reference
	× 10^{-4}	
Chl *a* in acetone	0.5	(26)
Chl *b* in acetone	0.6	(26)
Thylakoid developed under intermittent light	5	
LHCP *in vivo* after		
8 h of continuous light	48	
12 h of continuous light	58	
of chloroplasts grown in a greenhouse	50	(10)
LHCP purified		
Nonaggregated	5	(13)
Aggregated	60	(13)

Chloroplasts with primary thylakoids displayed a CD signal which can be interpreted as a sum of the CD signals obtained with the core-complexes (CP 1 and CP 2) of the two photosystems, neither of which contain Chl *b* (10). If this is so, the spectrum contains at least two partially overlapping exciton signals.

In the subsequent developmental stage triggered by continuous light, CD changes of chloroplast membranes can be characterized by the progressive appearance of a large CD signal from Chl *a* and of a band attributable to Chl *b*. After a period of continuous illumination, a CD spectrum characteristic of a mature chloroplast is seen.

Intensity differences in CD expressed as differential absorption

could arise from the different nature of optical activity in primary and maturing thylakoids. The small differential absorption of primary thylakoids can originate from exciton interaction among Chl electronic transition moments (27).

The very high intensity of the CD measured with native LHCP *in situ* or with extracted LHCP aggregated by salts (13) implies a more complex organization than that of the photosystems. The CD signal of the LHCP *in vivo* is of nonconservative type with the longest wavelength positive and with a much larger area than the area of negative band. These features together with the differential absorbance approximately 100 times higher than that of the pigment in solution are reminiscent of the CD signal of bacterial reaction center complexes (22) and of high mol wt chlide Chl(ide)-apomyoglobin (23). The CD signals of LHCP *in vivo* and of purified LHCP aggregated *in vitro* are similar to each other in magnitude and wavelength dependence but they display opposite signs (13) which shows the lack of a complete correspondence between the organization of these two complexes.

As to the structural pattern of LHCP aggregates and other Chl(ide)-protein complexes, no detailed model can be offered so far (23). A common feature is the large proportion of α-helices contained by the protein component (19, 21). Such proteins can assemble to multimeric liquid crystal-like structures (16, 26) exhibiting large CD signals influenced by the optical properties of the helical host molecule, pitch length and the tilt of the pigment molecules embedded (15). The x-ray diffraction and freeze-etching studies on the organization of the extracted LHCP aggregated by salts have shown the existence of macromolecular helices incorporating six to seven molecules of 'monomeric' LHCP (18). Although the artificial LHCP aggregate might be somewhat different from the native aggregate, the latter may also contain liquid crystal-like regions. This textural organization is flexible and sensitive to the membrane environment as regards electric field, temperature, and pH (26), which can change conformation conditions for membrane phosphorylation, the event which leads to the regulation of quantum distribution between the photosystems by LHCP (5, 6).

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