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Light Regulation of Photosynthetic Membrane Structure, Organization, and Function

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The light environment during plant growth determines the structural and functional properties of higher plant chloroplasts, thus revealing a dynamically regulated developmental system. Pisum sativum plants growing under intermittent illumination showed chloroplasts with fully functional photosystem (PS) II and PSI reaction centers that lacked the peripheral chlorophyll (Chl) *a/b* and Chl *a* light-harvesting complexes (LHC), respectively. The results suggest a light flux differential threshold regulation in the biosynthesis of the photosystem core and peripheral antenna complexes. Sun-adapted species and plants growing under far-red-depleted illumination showed grana stacks composed of few (3–5) thylakoids connected with long intergrana (stroma) thylakoids. They had a PSII/PSI reaction center ratio in the range 1.3–1.9. Shade-adapted species and plants growing under far-red-enriched illumination showed large grana stacks composed of several thylakoids, often extending across the entire chloroplast body, and short intergrana stroma thylakoids. They had a higher PSII/PSI reaction center ratio, in the range of 2.2–4.0. Thus, the relative extent of grana and stroma thylakoid formation corresponds with the relative amounts of PSII and PSI in the chloroplast, respectively. The structural and functional adaptation of the photosynthetic membrane system in response to the quality of illumination involves mainly a control on the rate of PSII and PSI complex biosynthesis.

Key words: photosystem development, chloroplast structure, chloroplast function, photosynthetic unit, gene expression, regulation

Our understanding of the photosynthetic membrane structural and functional organization has changed substantially over the last three years. As recently as 1980, it was commonly assumed that all of the chloroplast photosystem (PS) II and most of PSI reaction center complexes were located in the membrane of the grana partition region, in stoichiometrically equal amounts. A remainder of PSI was assumed to occur in stroma-exposed thylakoids [1,2]. This picture of the photosynthetic membrane structural and functional organization has changed dramatically. First, it was recognized that, contrary to the common prejudice dictated by the Z-scheme, there is no stoichiometric equality between PSII and PSI complexes in higher plant chloro-

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plasts [3–6]. Other studies provided evidence that PSI is totally excluded from the membrane of the grana partition region and is located exclusively in stroma thylakoids [7–9]. Independently, evidence was obtained for a structural and functional differentiation of PSII into two types, $PSII_\alpha$ and $PSII_\beta$, shown to be localized in the membrane of the grana partition region and in the stroma-exposed thylakoids, respectively [9–14]. Only $PSII_\alpha$ in the grana partitions contained the full complement of the Chl *a/b* LHC. On the basis of kinetic evidence, $PSII_\beta$ was shown to contain only about half the number of chlorophyll molecules in its LH antenna [14,15]. A similar functional differentiation for PSI was not detected, suggesting that PSI occurs as a uniform (homogeneous) population in the higher plant chloroplasts [9,13–16].

The formation of the photosynthetic apparatus can be monitored after exposure of etiolated leaves to continuous or intermittent illumination [17–19]. Under conditions of intermittent illumination, the rate of chlorophyll biosynthesis is light-limited, thus limiting the development of the chloroplast membranes and that of the photosystems. Intermittent illumination plastids show a significantly smaller antenna size for its photochemical reaction centers [18,19], synthesize selectively Chl *a*, and lack a major portion of the Chl *a/b* LHC and of grana [17–20]. Thus, intermittent light plastids are an attractive experimental material in which to probe developmental and gene expression properties of the photosynthetic apparatus.

Different environmental light conditions are also known to cause structural and functional changes in the photosynthetic apparatus [4,21–23]. The case of sun and shade adaptation of higher plant chloroplasts has attracted a lot of attention [4,23], since it provides a classic physiological example of light-quality-controlled gene expression and photosynthetic apparatus organization.

In the present work, the effect of light on chloroplast development and photosynthetic membrane organization was tested. Pea plants were grown under different intermittent light conditions and also under sun-/shade-simulated conditions in the laboratory. Obligate shade species were also tested for structural-functional properties of their photosynthetic membranes. The experimental approach taken involved spectrophotometric and kinetic analyses. We correlated structural parameters, such as the extent of grana formation under the different light conditions, with the relative amounts of PSII and PSI reaction centers present. We also measured the functional photosynthetic unit size of each photosystem under the different developmental conditions.

MATERIALS AND METHODS

Pisum sativum, L (pea) var Alaska plants were cultivated in the greenhouse under natural light conditions and also in a growth chamber under controlled light conditions. Intermittent light plastids were obtained by germinating Pisum plants in the dark for 5 days followed by 5 days of light-dark cycles (2 min L + 98 min D or 2 min L + 48 min D). For the intermittent light plastids, illumination was provided by both cool-white fluorescent and incandescent light bulbs. Pisum plants were also grown under continuous light of varying qualities. Far-red-deficient illumination was obtained by a system of 30W GE cool-white fluorescence tubes, while far-red-enriched light was obtained by 50W GE incandescent lamps. In both cases, the light intensities in the 400–700-nm region were approximately 8 mol quanta m^{-2} per day.

The obligate shade species Polystichum munutum Presl (sword fern) was grown in a shaded habitat at an average intensity of 3 mol quanta m^{-2} per day.

Chloroplasts were isolated and resuspended in a 50 mM Tricine buffer, pH 7.8, also containing 400 mM sucrose, 5 mM $MgCl_2$ and 10 mM NaCl. Chlorophyll concentrations and the Chl *a*/Chl *b* ratios were determined by computer analysis of the absorbance spectra of chloroplast extracts in 80°/o (v/v) acetone from the equations given by Arnon [24].

Chloroplast fluorescence and absorbance difference measurements were performed with a laboratory-constructed modulated split-beam difference spectrophotometer [25]. The optical pathlength of the cuvette for the measuring beam was 1.4 mm, and for the actinic beam it was 1.0 mm. Actinic excitation was provided by green light of uniform field transmitted by a combination of CS 4-96 and CS 3-69 Corning glass filters. Green light was selected in order to provide as equal as possible excitation of both Chl *a* and Chl *b* molecules [15].

The absolute concentrations of PSII and PSI reaction center complexes in the various samples were estimated from quantitative measurements of the light-induced absorbance change at 320 and 700 nm for Q and P700, respectively [3]. A relative measure of the chlorophyll (*a* + *b*) antenna size of PSI, $PSII_\alpha$, and $PSII_\beta$ was obtained from the rate of light absorption by each photosystem [13–15].

The rate of light absorption by PSII was determined under limiting-light conditions from the analysis of the area over the fluorescence induction curve of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and hydroxylamine-treated chloroplasts [11]. The rate of light absorption by PSI was determined from the analysis of the kinetics of the absorbance change at 700 nm, under the same excitation conditions [15]. To eliminate secondary electron transport to $P700^+$ from plastocyanin, cytochrome *f*, and plastoquinone, the chloroplasts were pretreated with potassium cyanide, which blocks plastocyanin function [16]. Chloroplasts were preincubated for 20 min in the presence of 25 mM KCN in a medium containing 20 mM Tricine (pH 7.8), 10 mM NaCl, 5 mM $MgCl_2$, and 200 mM sucrose. Following the KCN pretreatment, the chloroplasts were washed and resuspended in the isolation buffer.

Under the above experimental conditions, the kinetics of P700 photooxidation were exponential functions of time (eg, Fig. 1). Any interference in the kinetics from charge recombinations was ruled out since a short (3 μsec) flash caused irreversible absorbance change at 700 nm. The photooxidized P700 was restored very slowly in the dark (on the order of minutes) in the presence of DCMU and when methylviologen was used as the terminal electron acceptor [15,16].

RESULTS

Intermittent Light Plastids

In the first group of experiments, we compared the photochemical apparatus organization of pea control chloroplasts with that of intermittent light plastids developed under light-dark cycles of variable duration. Table I shows that plastids developed under 2 min L + 98 min D were devoid of Chl *b* (Chl *a*/Chl *b* > 20). Electron microscopy revealed that such plastids were also devoid of grana or appressed lamellae [20]. Plastids developed under 2 min L + 48 min D were able to synthesize limited amounts of the Chl *a/b* LHC, and this was evidenced by the lower Chl *a*/Chl *b*

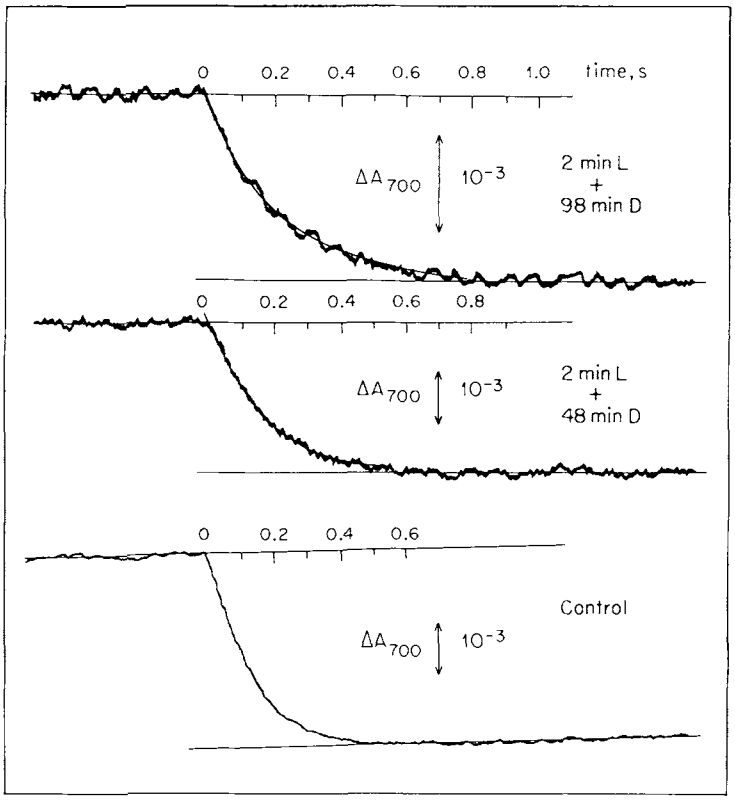

Fig. 1. The time course of the absorbance change at 700 nm monitored in the presence of 25 μM DCMU and 200 μM methyl-viologen. Green actinic illumination of uniform field came on at zero time. The samples contained the following Chl (*a* + *b*) concentrations: 52 μM (2 min L + 98 min D), 100 μM (2 min L + 48 min D), and 260 μM (control). Note the different time course of the three traces.

TABLE I. Photosynthetic Parameters of Intermittent Light Developing Pisum Chloroplasts*

	Chl *a* / Chl *b*	Chl / P700	Chl / Q	Q / P700	$PSII_\beta$ (%)	N_α	N_β	N_{P700}
2 min L + 98 min D	>20	250 ± 15	85 ± 15	2.9 ± 0. 6	100 ± 10	--	56 ± 8	90 ± 10
2 min L + 48 min D	6–8	310 ± 15	155 ± 20	2.0 ± 0. 3	58 ± 10	170 ± 20	28 ± 5	136 ± 15
Control	~2.7	635 ± 30	380 ± 20	1.7 ± 0.15	20 ± 5	280 ± 20	90 ± 15	220 ± 20

*The values reported represent the average of three to five measurements. Intermittent light pea plastids were grown in a growth chamber under the specified white light regime. Mature pea chloroplasts (control) were isolated from greenhouse peas. Chl/P700 and Chl/Q is the total Chl (*a* + *b*) present per P700 and Q, respectively. N is the No. of Chl (*a* + *b*) molecules specifically associated with a photosystem.

ratio of 6–8 determined in such preparations (Table I). They still lacked fully developed grana; nevertheless, they exhibited thylakoid membrane pairings; ie, membrane areas where two individual thylakoids were stacked. Clearly, the difference between the less developed (2 min L + 98 min D) and the more developed (2 min L + 48 min D) intermittent light plastids is due to the total flux of light they receive, apparently because the total flux of light determines the rate of chlorophyll biosynthesis and probably that of other thylakoid membrane components. In pea plants grown in the greenhouse under physiological light conditions, the rate of chlorophyll biosynthesis is not limiting and such chloroplasts have fully developed grana and a Chl *a*/Chl *b* = 2.7 (see Table I).

We determined the total chlorophyll per P700 and total chlorophyll per Q ratios for the less developed, more developed and control pea chloroplast samples. The Chl/P700 and Chl/Q ratio is a measure of the number of chlorophyll molecules present, and Table I shows that the chlorophyll content increased progressively with the developmental stage of the plastids. Table I also shows that the Chl/P700 and Chl/Q values for a sample do not match, suggesting uneven amounts of PSII(Q) and PSI(P700) complexes. A measure of the PSII/PSI reaction center ratio was obtained from the Q/P700 ratio in the various samples (Table I). There was an excess amount of PSII in the less developed chloroplasts (Q/P700 ~3) as compared to more developed chloroplasts (Q/P700 ~2). In the fully mature chloroplast sample there was still more PSII present (Q/P700 ~1.7), although the relative number was about half of that determined for the less developed chloroplasts. One possible interpretation of this phenomenon is that in the early stages of development the rate of system II component biosynthesis exceeds that of system I. This may be dictated by the structural organization of higher plant chloroplasts where PSII complexes are eventually incorporated into the membrane of the grana partition regions, whereas PSI complexes remain in stroma-exposed thylakoids [7–9]. Thus, the more elaborate developmental process of the grana membranes may require an early biosynthesis of the corresponding structural and functional components.

Although the Chl/P700 and Chl/Q values gave a measure of the total amount of chlorophyll present, they cannot provide information on the functional photosynthetic unit size of either photosystem because they do not specify how many Chl molecules of the total are associated exclusively with PSI and how many are associated with PSII [4]. To determine the number of Chl molecules functionally associated with each photosystem, we have developed a kinetic method based on the rate of light absorption by the respective reaction centers [13–15]. The rate of light absorption by a reaction center depends on the actinic light intensity, on the number of chlorophyll molecules functionally associated with the reaction center and on the quantum yield of photochemistry for the particular photoreaction [15]. The quantum yield of system II and system I photochemistry approaches unity in mature chloroplasts [26–29]. This is probably also true for developing chloroplasts, since the light-harvesting antenna size is smaller and thus the average excitation migration distance to the reaction center is shorter. Thus, under conditions of continuous actinic illumination of constant intensity, differences in the rate of light absorption by a reaction center can be translated into different light-harvesting chlorophyll antennae.

The rate of light absorption by the reaction centers of PSI was measured with plastocyanin-inhibited chloroplasts in the presence of methyl-viologen [16]. Figure 1 shows the kinetics of P700 photooxidation upon continuous illumination of the less

developed plastids (2 min L + 98 min D), of the more developed plastids (2 min L + 48 min D), and mature pea chloroplasts (control). Clearly, under our experimental conditions, the rate of P700 photooxidation depends on the rate of light absorption by PSI, and it is faster for the more developed chloroplasts than for the less developed chloroplasts but slower than that of the mature chloroplasts. Different rates of light absorption by PSI reflect differences in the PSI functional antenna size for the three types of chloroplasts.

More quantitative information can be extracted from the kinetic curves of Figure 1 by plotting them on a semilogarithmic plot. Such analysis is shown in Figure 2. All three P700 photooxidation curves appear as single straight lines in the semilogarithmic presentation, suggesting that within each sample there is a uniform population of PSI complexes; ie, all PSI complexes within a sample have the same functional antenna size. The slope of the straight lines differs among the three samples, however. The slope, expressed in units of "per second" is a direct measure of the number of photons per second arriving at the respective reaction center. This number will be used to calculate the number N of chlorophyll molecules transferring excitation energy to P700 (see below). Figure 2 also shows that progressive with the developmental stage of the chloroplast, the slope of the semilogarithmic lines becomes steeper. It is implied that all chlorophyll molecules that are available to PSI in the various developmental stages are evenly distributed among the existing PSI complexes.

The developmental properties of PSII are considerably different from those of PSI. Figure 3 shows a measurement of the rate of light absorption by PSII reaction centers, monitored by the variable portion of the fluorescence induction kinetics in the presence of DCMU. In the early stages of PSII development (2 min L + 98 min D) the fluorescence induction kinetics are a slow single exponential function of time (see Fig. 4, solid circles) suggesting a uniform population of PSII core complexes of small light-harvesting antenna size. Upon the biosynthesis of small amounts of the Chl *a/b* LHC (2 min L + 48 min D, Fig. 3) the fluorescence induction occurs with biphasic kinetics (see also Fig. 4, open circles). We have determined that the amplitude of the fast α-phase originated from about 40% of the total PSII present, suggesting that under the developmental conditions used in this experiment, about 40% of the PSII reaction centers received a complement of the Chl *a/b* LHC and thus increased the size of the functional LH antenna ($PSII_\alpha$), while the remaining 60% did not receive the Chl *a/b* LHC complement, and as such they are composed of a small core Chl *a* antenna only ($PSII_\beta$). Thus, under conditions of limiting Chl *a/b* LHC biosynthesis, the available Chl *a/b* LHC are not evenly distributed among the existing PSII units. This constitutes a significant photochemical unit developmental difference between PSII and PSI. In mature pea chloroplasts, grown under conditions when the biosynthesis of the Chl *a/b* LHC is not limited (Fig. 3, control; Fig. 4, crosses), about 80% of all PSII complexes contain the Chl *a/b* LHC complement in their antenna, while only a minor portion of about 20% appear lacking it. Table I shows the percentage of $PSII_\beta$, ie, the fraction of PSII lacking the Chl *a/b* LHC under the various chloroplast developmental conditions.

A comparison of the relative rates of light absorption by the three photosystems is given in Figure 5 for the more developed pea chloroplasts (2 min L + 48 min D). The value of the various constants for light absorption (K_α, K_β, and K_{P700}) is defined from the respective slopes and it is directly proportional to the number, N, of chlorophyll molecules transferring excitation energy to the particular photosystem [15]. The determination of the number of chlorophyll molecules transferring excita-

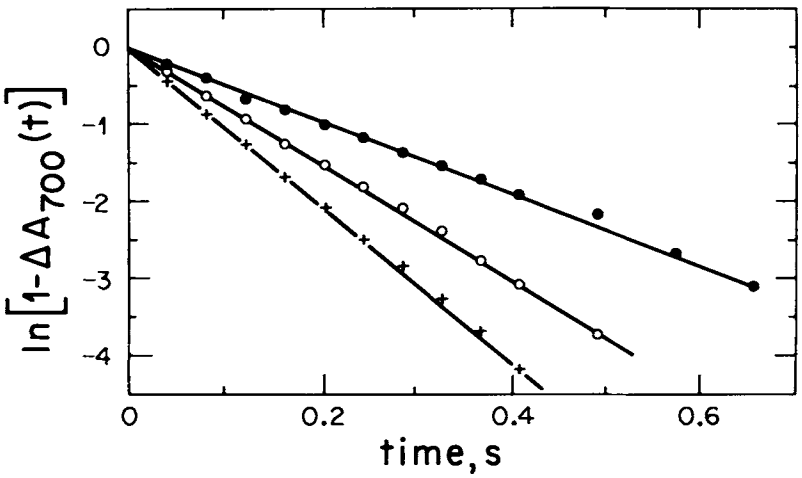

Fig. 2. A semilogarithmic plot of the kinetics of ΔA_{700} showing the monophasic exponential nature of P700 photooxidation. The slope of the straight line defines the rate constant K_{P700} of light absorption by PSI. Pea thylakoids were greened under 2 min L + 98 min D (solid circles), 2 min L + 48 min D (open circles) or in the greenhouse under physiological light conditions (crosses).

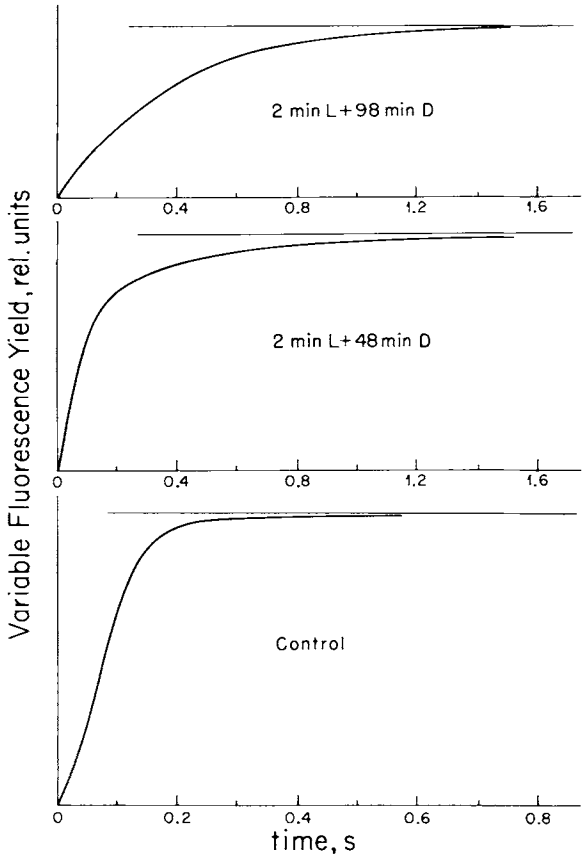

Fig. 3. The variable fluorescence induction kinetics of isolated pea chloroplast thylakoids measured in the presence of 25 μM DCMU. Chlorophyll concentration was 50 μM. Note the different shapes of the three kinetic curves.

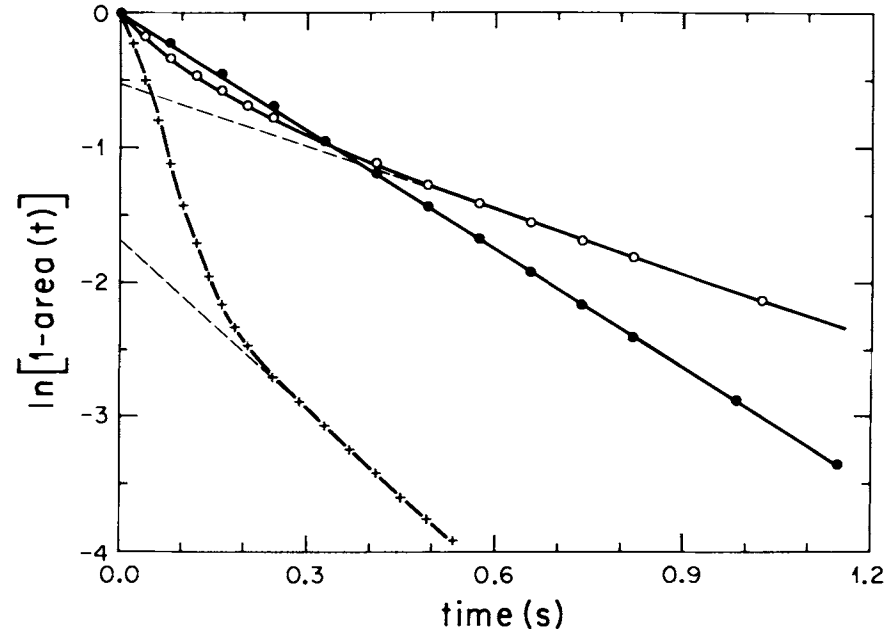

Fig. 4. A semilogarithmic plot of the kinetics of the area over the fluorescence induction curves shown in Figure 3. Note the monophasic function of time obtained from plastids greened under 2 min L + 98 min D (solid circles). In the biphasic curves (open circles, crosses), the slope of the linear β-phase (dashed lines), defined the rate constant K_α of light absorption by $PSII_\beta$. The rate constant K_α of light absorption by $PSII_\alpha$ was determined from the fast phase after subtraction of the contribution by the slow phase [11].

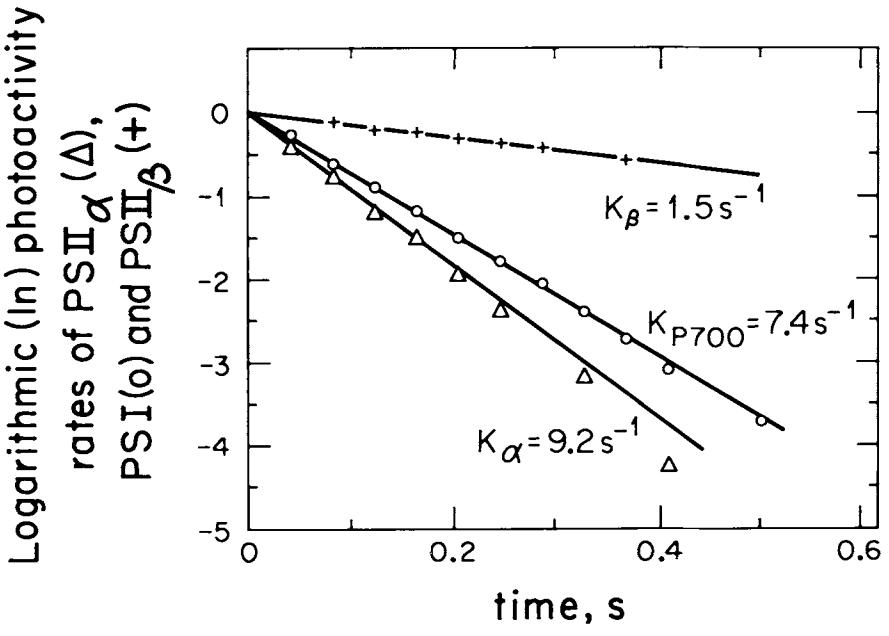

Fig. 5. Semilogarithmic plots of the primary photoactivity kinetics of PSIIα ($\triangle$), PSI (O), and $PSII_\beta$ (+) from intermittent-light-grown plastids (2 min L + 48 min D).

tion to each photosystem was implemented by the solution of the following system of equations:

$$\frac{\text{Chl}}{\text{PSI}} = \frac{\text{PSII}_\alpha}{\text{PSI}} N_\alpha + \frac{\text{PSII}}{\text{PSI}} N_\beta + N_{P700} \quad (1)$$

$$K_\alpha = c\ I\ N_\alpha \quad (2)$$

$$K_\beta = c\ I\ N_\beta \quad (3)$$

$$K_{P700} = c\ I\ N_{P700} \quad (4)$$

where Chl/PSI is the ratio of total Chl (a + b) per PSI reaction center, I is the actinic light intensity, and c is a proportionality constant depending on the quantum yield of photochemistry at each photosystem. Assuming the same quantum yield of photochemistry at each photosystem for a set of developmental conditions, we have solved the system of equations [1–4] to obtain the number, N, of chlorophyll molecules transferring excitation energy to a specific reaction center. Table I shows the result from such an approach: There are only 90 Chl molecules exclusively associated with PSI in the less developed chloroplasts (2 min L + 98 min D), but this number increases to 220 Chl molecules in the mature pea chloroplasts (control). Similarly, a uniform population of PSII antennae with 56 Chl molecules are found in the less-developed chloroplasts. This number increases to N_α = 170 in the more developed chloroplasts (2 min L + 48 min D), while a large $PSII_\beta$ population with N_β = 28 also appears. In the control pea chloroplasts, N_α = 280 and N_β = 90 constitute the steady-state antenna size of $PSII_\alpha$ and $PSII_\beta$, respectively, under physiological light growth conditions.

Sun-Shade Adaptation

In the second group of experiments, we tested the effect of far-red (FR)-enriched illumination on chloroplast development. The balance between red and far-red light during plant growth is very important because it determines the activation state of phytochrome. It is also important because FR light, especially that in the 690–710-nm region, is absorbed exclusively by PSI. Thus, the balance between red and far-red light will determine the balance of excitation energy delivered to PSII and PSI reaction centers. Figure 6 shows that pea chloroplasts developed under FR-depleted illumination show a photosynthetic membrane ultrastructure of thin and low-density grana in the chloroplast, interconnected by long stroma-exposed thylakoids. On the contrary, pea chloroplasts developed under FR-enriched illumination appear to have thicker grana of higher density, interconnected by very short stroma-exposed thylakoids (Fig. 7). Precisely the same thylakoid membrane ultrastructural differences are detected between chloroplasts from sun-adapted and shade-adapted plants [4,21–23,30]. By analogy to chloroplasts developed in a FR-enriched light environment, shade-adapted chloroplasts also show large grana stacks, sometimes spanning the entire chloroplast body, with short intergrana lamellae. A typical such example is shown in Figure 8 for a Polystichum munutum (sword fern) chloroplast. A point worth mentioning is that in shade-adapted chloroplasts, the massive grana provide a significant resistance to the microtome knife during thin sectioning. The result is that grana from shade chloroplasts invariably break to smaller pieces during sample preparation and grana

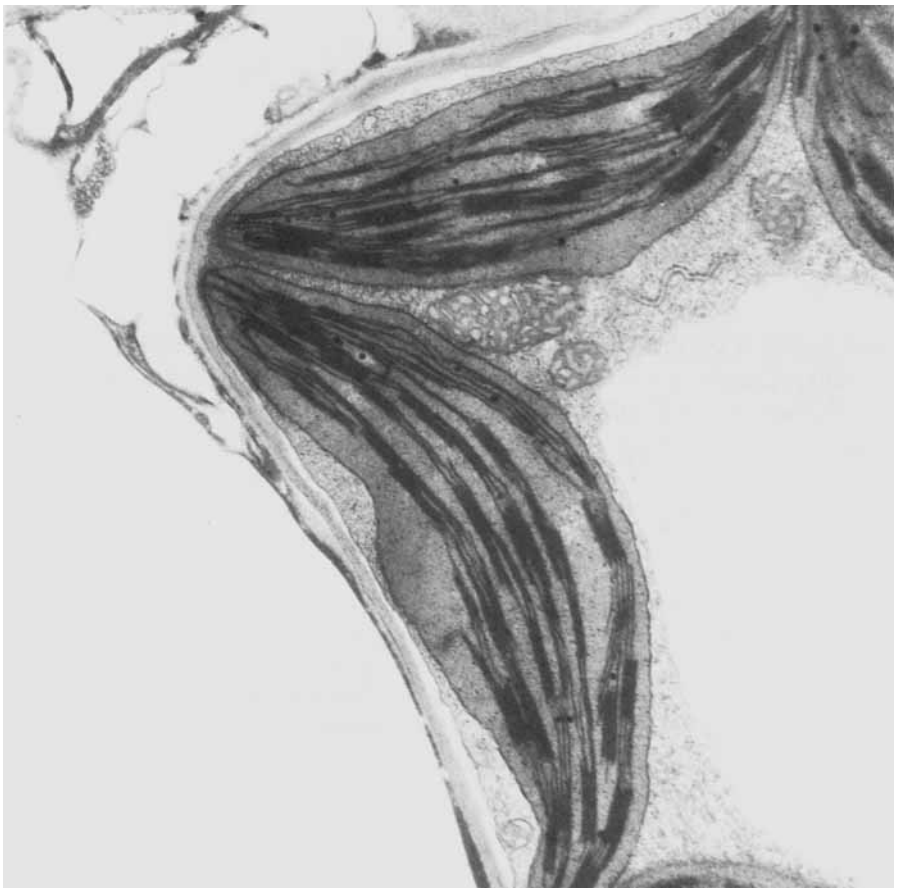

Fig. 6. Electron micrograph of a pea chloroplast cross section developed under far-red-deficient illumination. Note the thin grana stacks and the long stroma thylakoids interconnecting the grana. ×19,000.

portions may be entirely displaced. The analogy between the light environment artificially enriched in FR and the shade ecotype is not accidental, since a shade ecotype is known to be significantly enriched in FR but depleted in both red and blue irradiation [21,31]. We looked into the question of whether membrane structural differences induced by light-quality variations during plant growth are reflected in the functional and organizational properties of the photochemical apparatus.

Table II shows that the relative extent of grana formation is reflected in the Chl *a*/Chl *b* ratio. Since Chl *b* is located mostly in the membrane of the grana partition regions [3,7–9], the Chl *a*/Chl *b* ratio may serve as a biochemical test for the extent of grana formation. In this respect, it is observed that Polystichum chloroplasts showed the lowest Chl *a*/Chl *b* ratio (2.4) and pea chloroplasts developed in the absence of FR illumination (small grana, long stroma-exposed thylakoids) showed the highest Chl *a*/Chl *b* ratio (see Table II). Pea chloroplasts developed under FR-

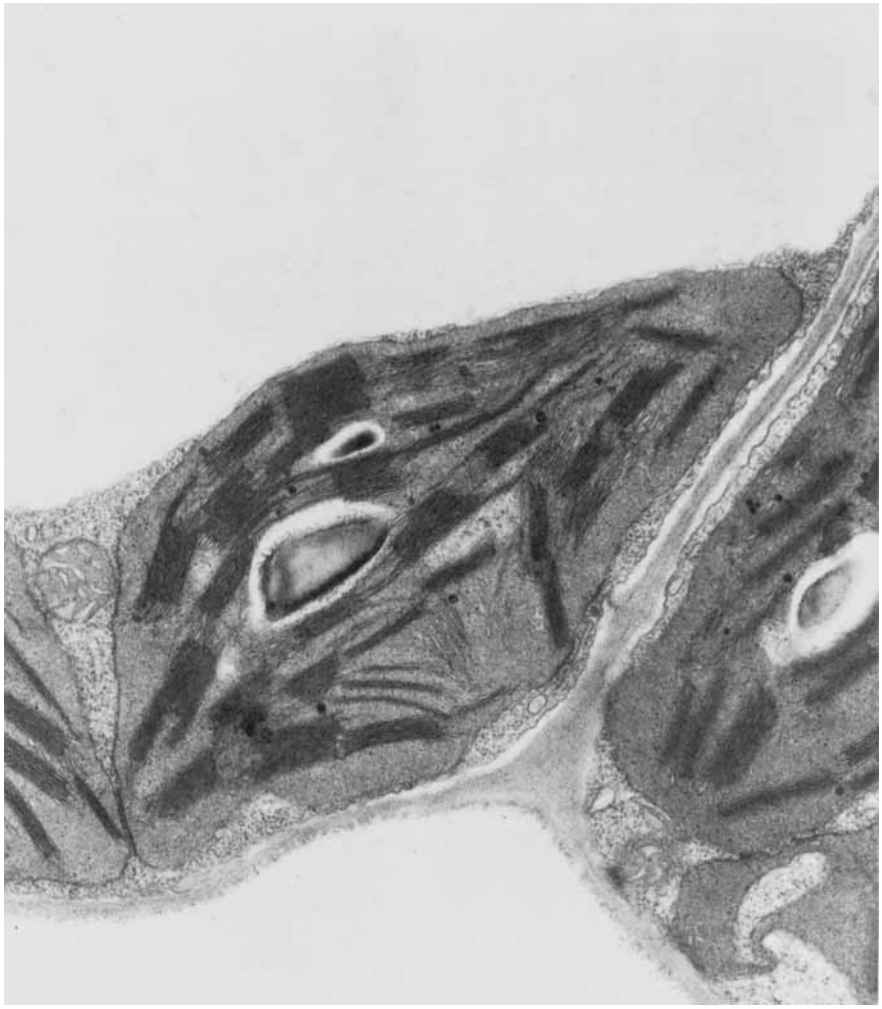

Fig. 7. Electron micrograph of a pea chloroplast cross section developed under far-red-enriched illumination. Note the thick grana stacks and the short stroma thylakoids that interconnect grana. ×19,000.

enriched illumination showed consistently greater Chl/P700 but lower Chl/Q values from chloroplasts developed in FR-depleted illumination, suggesting an effect of FR illumination on the PSII/PSI reaction center ratio of the chloroplasts. Table II shows that the ratio of Q/P700 was low, about 1.3 ± 0.1, for the chloroplasts with the small grana and long stroma thylakoids (−FR), while it was high, about 2.2 ± 0.2, for the chloroplasts with the high grana density and short intergrana membranes (+FR). This stoichiometric imbalance between the two photosystems was further pronounced in the shade-adapted chloroplasts of Polystichum (Q/P700 = 3.4, see Table II). It appears, therefore, that the relative amounts of PSII and PSI reaction centers parallel the relative extent of grana and stroma thylakoid formation, respectively. This inter-

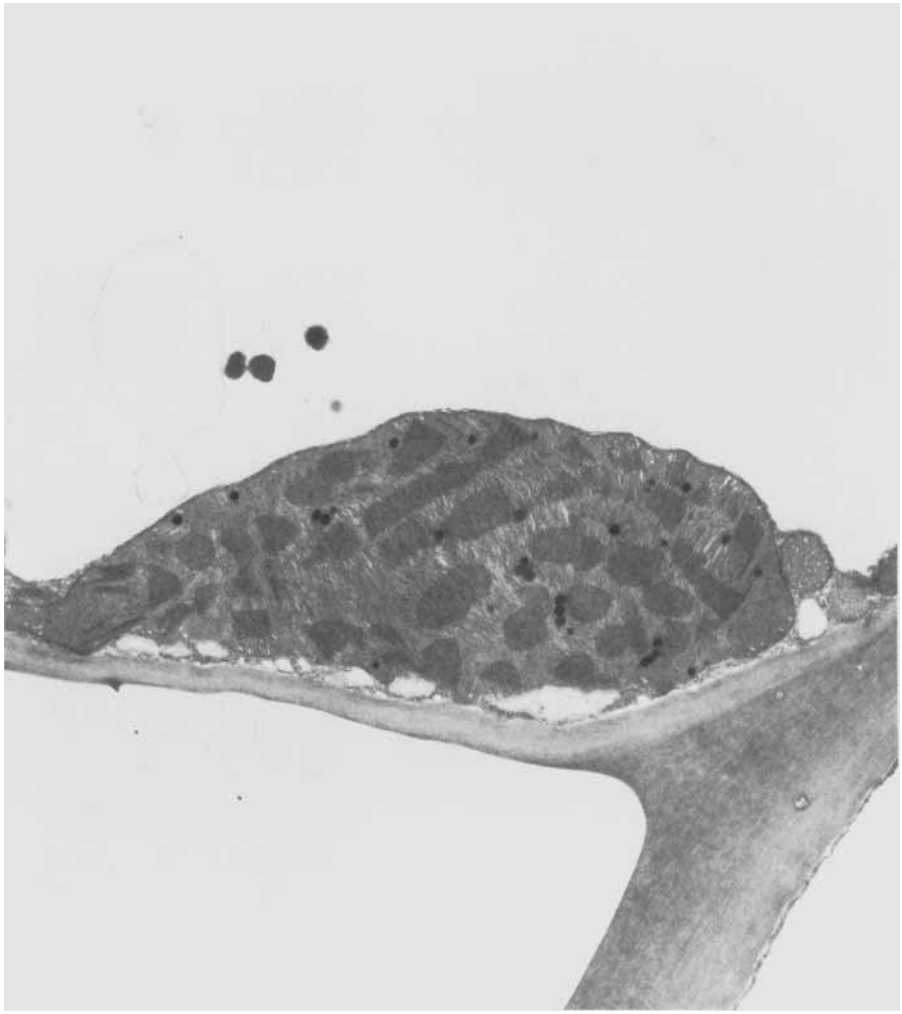

Fig. 8. Electron micrograph of a Polystichum chloroplast cross section showing the high density of thick grana that appear spanning the entire chloroplast body. Note also the short intergrana membranes. ×8,000.

pretation is in agreement with the hypothesis of the exclusive location of PSII in the membrane of the grana partition region and the exclusive location of PSI in stroma-exposed thlakoids [7–9]. They also argue for a regulatory light quality control on photosynthetic gene expression in higher plant chloroplasts.

It has been reported that one important plant strategy in the sun-shade adaptation is the modification of the photosynthetic unit size in order to favor excitation of PSII or PSI, depending on the conditions [32–34]. We tested the validity of this hypothesis by measuring the functional photosynthetic unit size of $PSII_{\alpha}$, $PSII_{\beta}$, and PSI (N_{α}, N_{β}, and N_{P700}) for pea chloroplasts grown under FR-enriched and FR-depleted illumination. Figure 9 shows the P700 photooxidation kinetics of pea chloroplasts grown under the two different light-quality conditions. Figure 10 compares the slopes (ie,

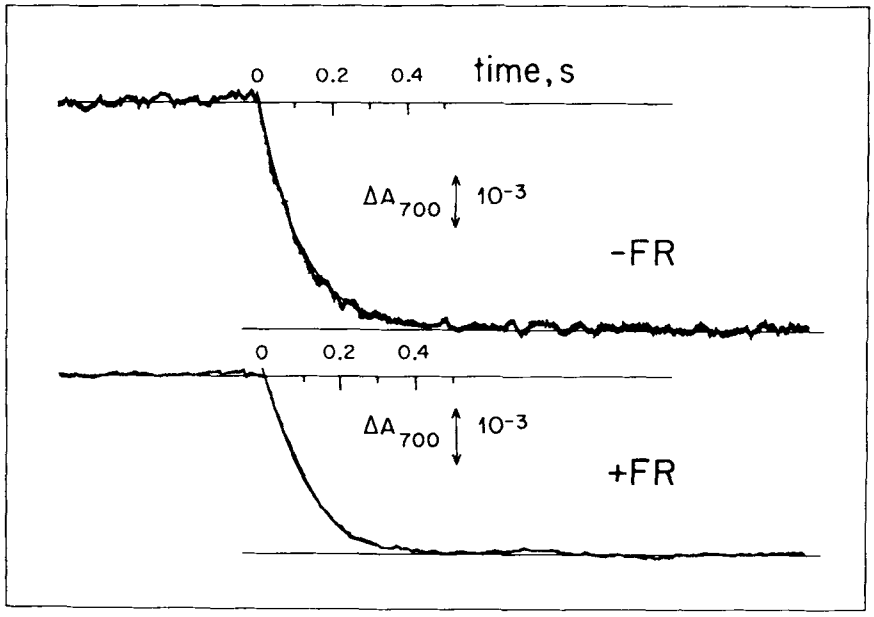

Fig. 9. Kinetics of P700 photooxidation in isolated pea chloroplasts developed under far-red-deficient (−FR) or far-red-enriched illumination (+FR). The reaction mixture contained 25 μM Chl (a + b).

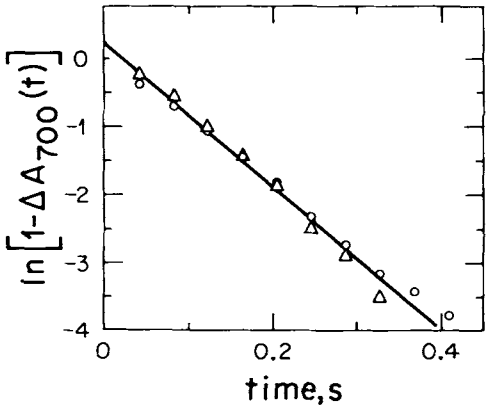

Fig. 10. Semilogarithmic plots of the kinetics of P700 photooxidation from Figure 9. Pea chloroplasts were greened under far-red-deficient (circles) and far-red-enriched (triangles) illumination.

the rates of light absorption by P700 of the two kinetic curves in the semilogarithmic presentation). Within experimental error, the slope of the straight lines in the semilogarithmic presentation are identical, suggesting equal functional photosynthetic unit size of PSI in the +FR and −FR chloroplast samples. A similar analysis for PSII revealed that the rate constants K_α and K_β were very similar in pea chloroplasts grown under FR-enriched or FR-deficient illumination (not shown).

TABLE II. Photosynthetic Parameters of Sun/Shade-Adapted Chloroplasts*

	Chl *a* / Chl *b*	Chl / P700	Chl / Q	Q / P700	N_α	N_β	N_{P700}
−FR (Fluorescent)	2.9 ± 0.16	550 ± 30	430 ± 20	1.3 ± 0.1	300 ± 20	95 ± 30	225 ± 25
+FR (Incandescent)	2.7 ± 0.14	735 ± 30	330 ± 20	2.2 ± 0.2	265 ± 20	80 ± 15	215 ± 15
Polystichum (fern)	2.4 ± 0. 1	850 ± 40	250 ± 50	3.4 ± 0.7	210 ± 30	--	100 ± 20

*The values reported represent the average of three to five measurements. Pea plants were grown under far-red-depleted and far-red-enriched light in a growth chamber (see Materials and Methods).

Table II shows that in spite of the large PSII/PSI stoichiometric ratio change in pea chloroplasts, the number of chlorophyll molecules ($N_\alpha = 280 \pm 20$, $N_\beta = 90 \pm 30$, and $N_{P700} = 220 \pm 20$) transferring excitation energy to $PSII_\alpha$, $PSII_\beta$, and PSI remained practically unchanged. Thus, the main plant strategy in the sun-shade adaptation is to change the relative number of $PSII_\alpha$, $PSII_\beta$, and PSI complexes that are synthesized under the particular conditions. However, the photosynthetic unit size of each individual complex remained unaltered.

DISCUSSION

The present work contributed further evidence suggesting that higher plant chloroplasts are dynamic organelles in constant interaction with the light environment. Experimental and/or environmental variations in the light conditions during plant growth may cause differential expression of organelle and nuclear genes coding for the various chloroplast components. Thus, in intermittent light-grown plants, when the total flux of light is below a certain threshold, nuclear genes coding for the PSII-Chl *a/b* LHC and for the PSI-Chl *a* peripheral antenna are not expressed. The resulting intermittent light plastids do possess, however, functional PSII and PSI reaction centers equipped with minimal core-Chl *a* antenna, suggesting that the biosynthesis and regulation of the PSII and PSI core complexes is subject to a different environmental and/or genetic control than that of their peripheral Chl-LHC. This evaluation is in agreement with the hypothesis that, unlike the peripheral Chl-LHC of PSII and PSI, the reaction center-core antenna complex of both photosystems is coded for and regulated by organelle genes [35].

Another level of regulation in gene expression is manifested when plants are grown under different qualities of light. An apparent alteration in the balance of red and far-red illumination causes substantial structural, organizational and functional changes in higher plant chloroplasts. These are expressed in the form of variable extent of grana vs stroma-exposed thylakoids and also variable relative amounts of PSII and PSI complexes present. It is not clear whether such structural and functional changes are directly mediated by phytochrome. It has been suggested [36,37] that phytochrome activation by red light results in greater mRNA levels for the Chl *a/b* LHC. Under our conditions, however, the absence of far-red light caused extensive stroma-exposed thylakoid formation, small grana stacks, and lower relative amounts of the Chl *a/b* LHC containing $PSII_\alpha$. A different approach in evaluating the light quality effect on chloroplast component biosynthesis and organization is to observe that under far-red-enriched illumination PSI receives excitation energy at a much higher rate than PSII. Then, the light quality-induced changes in the chloroplast

structure and function occur in response to the imbalance of light input between PSII and PSI. In this respect, our spectrophotometric and kinetic analyses revealed that the functional antenna size of individual PSII and PSI complexes does not change. It is the relative number of these complexes that can vary, suggesting a mechanism for the differential regulation and/or expression of genes coding for PSII and PSI complexes. Clearly, more work in this direction is required to elucidate the details of such regulation.

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