Biochimica et Biophysica Acta 932 (1988) 130–137
Elsevier

BBA 42688

Chlorophyll *b* deficiency in soybean mutants. I. Effects on photosystem stoichiometry and chlorophyll antenna size

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(Received 19 June 1987)
(Revised manuscript received 21 September 1987)

Key words: Chlorophyll antenna size; Photosystem II heterogeneity; Photosystem stoichiometry; Light harvesting complex; (Soybean chloroplast)

The stoichiometry of photochemical reaction centers and the chlorophyll (Chl) antenna size of the photosystems is correlated in thylakoid membranes from soybean wild-type and two Chl-*b*-deficient mutants (y_9y_9 and $Y_{11}Y_{11}$). Thylakoids from mutant plants displayed two characteristic changes in the organization of the photosystems. (a) The light-harvesting Chl antenna of PS II_α was lowered from about 250 Chl $a + b$ in the wild type to about 150 Chl in y_9y_9, and 140 Chl molecules in the $Y_{11}y_{11}$ mutant. That of PS II_β remained invariant at about 120 Chl $a + b$ molecules in the wild type and y_9y_9 but was slightly smaller in the $Y_{11}y_{11}$. The antenna of PS I was lowered from 210 Chl $a + b$ in the wild type to about 160–170 Chl molecules in the two mutants. (b) The photosystem stoichiometry was altered substantially from PS II_α/PS II_β/PS I = 1.5:0.4:1.0 in the wild type, to 1.6:0.9:1.0 in the y_9y_9, and 1.6:1.2:1.0 in the $Y_{11}y_{11}$ chloroplasts. Accumulation of PS II_β in the mutant thylakoids correlated with the degree of Chl *b* deficiency, suggesting that PS II_β is an intermediate stage in the development of the mature PS II complex. Moreover, higher PS II/PS I ratios in the thylakoid membrane of y_9y_9 and $Y_{11}y_{11}$ suggest a response of the plant in countering the smaller antenna size of PS II_α and establishing a balanced absorption of light by the two photosystems.

Introduction

Electron transport in the thylakoid membrane of higher plant chloroplasts is mediated by the photoreactions of Photosystem I (PS I) and Photosystem II (PS II). Each photosystem is defined as a membrane-bound complex composed of specialized chlorophyll (Chl) molecule(s), acting as the reaction center, and of specialized electron-acceptor molecules. Tightly bound to the reaction center is a 'core' Chl-*a*-containing complex which collects and transfers excitation energy to the photochemical reaction center. In addition, there is an accessory Chl-*a*- and Chl-*b*-containing light-harvesting complex (LHC). Each photosystem constitutes a structurally independent thylakoid membrane protein complex [1,2].

With respect to the chlorophyll antenna size and composition, PS I complexes constitute a uniform population in the thylakoid membrane of chloroplasts, evidenced by the monophasic P700 photooxidation kinetics [3,4]. However, PS II complexes exist in two distinct populations [5,6]. Het-

Abbreviations: Chl, chlorophyll; PS, Photosystem; Q_A, primary quinone of PS II; P700, reaction center of PS I; LHC, light-harvesting complex; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

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erogeneity arises from the bimodal distribution of the absorption cross-section of PS II, resulting in two populations of PS II centers, PS II_α and PS II_β. In wild-type chloroplasts, PS II_α accounts for about 75–80% of all PS II, displays a lower Chl *a*/Chl *b* ratio, larger Chl antenna size (about 230 Chl $a+b$ molecules) and is located in the appressed regions of the grana stacks forming clusters of three or four centers. In mature chloroplasts, PS II_β accounts for the remaining 20–25%, has a higher Chl *a*/Chl *b* ratio, smaller chlorophyll antenna size (about 120 Chl $a+b$ molecules) and forms isolated units in stroma-exposed chloroplast lamella regions [7]. Although PS II_β is functional in the process of charge separation and oxygen evolution, it displays a slow turnover of electrons from the reducing side, presumably because of low affinity and/or slow interaction with plastoquinone [8–10].

The origin and physiological significance of PS II_β is currently unknown. It has been proposed that PS II_β is involved in poising PS I for cyclic electron flow [11], is responsible for injecting protons directly into the ATP synthetase [12], or that it represents PS II in a developmental and/or repair stage [13]. Certain conditions appear to induce interconversion between the two types of PS II center, such as phosphorylation/dephosphorylation of the thylakoid membrane [14,15] and high-temperature treatment of chloroplasts [16].

In previous work, the photochemical apparatus organization and PS II heterogeneity in Chl-*b*-less barley chloroplasts was presented [13]: Chlorina f2 Chl-*b*-less mutant thylakoids lacked the differentiation of PS II into PS II_α and PS II_β. Instead, the development of PS II was arrested at a chlorophyll antenna size of about 50 Chl *a* molecules. The chlorina f2 mutant contains CP47, CP43 and CP29; however, it lacks the main LHC II polypeptides [17]. It was concluded that lack of Chl *b* prevented the assembly and incorporation of the LHC II components in the photosynthetic unit of PS II.

In the present work we investigated the development of the PS II antenna from the PS II stage of 50 Chl *a* molecules to PS II_α (230 Chl $a+b$ molecules). Thylakoid membranes from three soybean strains were utilized, i.e., wild-type, Clark y_9y_9 and Clark $Y_{11}y_{11}$. The mutants are deficient in Chl *b* in the early stages of leaf development only [18]. These mutants have high Chl *a*/Chl *b* ratios and lower amounts of LHC II polypeptides than the wild-type plants, but slowly accumulate Chl *b* upon development [19]. We measured antenna size of the photosystems and reaction center concentration in the thylakoid membrane of chloroplasts from leaves at different stages of development. The following distinct effects of Chl *b* deficiency on the organization and function of the photosystems were observed: (a) smaller functional chlorophyll antenna size for both photosystems, (b) higher PS II/PS I ratios, (c) enhanced PS II_β content. The accumulation of Chl *b* in these mutants is discussed in terms of a general model for the development of the light-harvesting antenna of PS II in higher plant chloroplasts.

Materials and Methods

Wild type soybean (Glycine max, Clark L1) and the Chl-*b*-deficient Clark y_9y_9 and Clark $Y_{11}y_{11}$ mutants were grown in vermiculite either in the greenhouse or in a growth chamber under continuous illumination provided by a combination of incandescent and fluorescent lamps. The light intensity in the growth chamber was 120 $\mu E \cdot m^{-2} \cdot s^{-1}$. The primary leaves of plants at different stages of growth were harvested. Thylakoid membranes were isolated at 0 °C as described [20], by grinding leaf segments for 40 s in a Waring Blendor using a 50 mM Tricine buffer (pH 7.8), containing 0.4 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$, 0.2% bovine serum albumin and 0.2% sodium ascorbate (isolation buffer). After removal of cell debris by centrifugation at $750 \times g$ for 1.5 min, thylakoid membranes were precipitated by centrifugation at $7000 \times g$ for 7 min. The pellet was resuspended in a small volume of a 50 mM Tricine buffer (pH 7.8) containing 10 mM NaCl and 5 mM $MgCl_2$ (hypotonic buffer). We used a variation of Arnon's equations [21] to determine total Chl concentration and the Chl *a*/Chl *b* ratio of chloroplast samples.

The concentration of the primary quinone electron acceptor, Q_A, of PS II was determined from the amplitude of the light-induced absorbance change at 320 nm using DCMU-treated chloro-

plasts suspended in the presence of 2.5 mM potassium ferricyanide. We used the procedure and equations of Pulles et al. [22] to determine flattening correction factors of about 1.3 at 320 nm, and applied a differential absorption coefficient of 13 $mM^{-1} \cdot cm^{-1}$ to the corrected absorbance difference at 320 nm [20].

The concentration of the reaction center P700 of PS I was determined from the amplitude of the light-induced absorbance change at 700 nm. The measurement was made using SDS-solubilized thylakoid membranes suspended in the presence of 2 mM sodium ascorbate and 200 μM methyl viologen. A differential absorption coefficient of 64 $mM^{-1} \cdot cm^{-1}$ was used for the calculation of P700 concentration [20]. The cuvette optical pathlength for all measurements was 0.18 cm.

The functional chlorophyll antenna size of PS II and PS I was estimated from the rate of light absorption by each photosystem under broad green actinic excitation [20] provided by a combination of CS 4-96 and CS 3-68 Corning filters. The actinic light intensity was 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ for all kinetic measurements. The rate of light absorption by PS I was determined from the rate of P700 photooxidation in KCN-treated thylakoids. The rate of light absorption by PS II was determined from the kinetic analysis of the fluorescence induction in DCMU-poisoned thylakoid samples. Assuming a similar quantum yield of primary photochemistry for PS II and PS I centers [23–26], we converted the rates of light absorption into functional chlorophyll antenna size by means of the equations presented [20,23,25,26]. Signal averaging and kinetic analyses were performed by an on-line Hewlett-Packard HP86B computer interfaced with a high-speed voltmeter.

Results

The expression of Chl *b* deficiency in the Clark y_9y_9 and Clark $Y_{11}y_{11}$ soybean mutants is developmentally regulated [18]. Fig. 1 shows the Chl *a*/Chl *b* ratio as a function of the primary leaf age in thylakoid membrane from wild-type (squares), Clark y_9y_9 (circles) and Clark $Y_{11}y_{11}$ (triangles) soybean plants grown either in the growth chamber under continuous illumination (Fig. 1A), or in the greenhouse (Fig. 1B). Wild-type soybean leaves had a constant Chl *a*/Chl *b* ratio of about 3.4 when grown in the growth chamber. The primary leaves of the mutants were deficient in Chl *b* when compared to wild type, as evidenced by the higher Chl *a*/Chl *b* ratios. The Clark $Y_{11}y_{11}$ mutant had a more pronounced Chl *b* deficiency (higher Chl *a*/Chl *b* ratio) than the Clark y_9y_9 mutant. The expression of Chl *b* deficiency in the two mutants depended strongly on the age of the leaf: younger leaves had higher Chl *a*/Chl *b* ratios than older leaves. Plants grown in the greenhouse (high light intensity, Fig. 1B) had overal higher Chl *a*/Chl *b* ratios and a slower acquisition of Chl *b* than plants grown in the growth chamber (low light intensity, Fig. 1A).

In order to investigate the effects of Chl *b* deficiency on the organization of the photosystems in the soybean mutants, we measured the Chl/P700 and Chl/Q_A ratio in thylakoids from plants at different stages of development. Fig. 2A shows the mol : mol ratio of Chl/P700 (open symbols) and of Chl/Q_A (solid symbols) as a function of the Chl *a*/Chl *b* ratio in wild-type (squares), Clark y_9y_9 (circles) and Clark $Y_{11}y_{11}$ (triangles) soybean chloroplast membranes. The data suggest that in the absence of Chl *b* (higher Chl *a*/Chl *b* ratios), there is significant enrichment in reaction centers, as evidenced by the lower Chl/P700 and

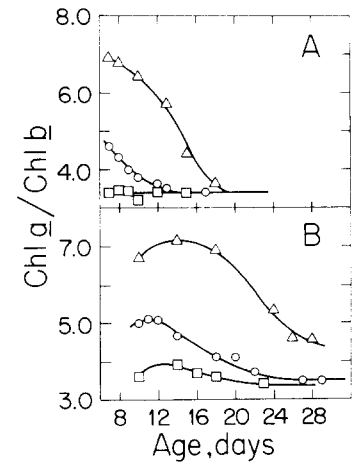

Fig. 1. The Chl *a*/Chl *b* ratio as a function of leaf age in thylakoids from wild-type (squares), y_9y_9 (circles) and $Y_{11}y_{11}$ (triangles) soybean plants grown either in a growth chamber under continuous illumination (A) or in the greenhouse (B). Only primary leaves were used for these measurements.

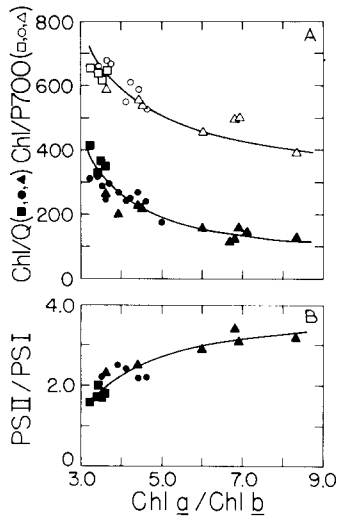

Fig. 2. (A) Chl/P700 (open symbols) and Chl/Q_A (solid symbols) as a function of the Chl *a*/Chl *b* ratio in thylakoid membranes from wild-type (squares), y_9y_9 (circles) and $Y_{11}y_{11}$ (triangles) soybean plants harvested at different stages of development. (B) The PS II/PS I reaction center ratio as a function of the Chl *a*/Chl *b* ratio in thylakoid membranes as above.

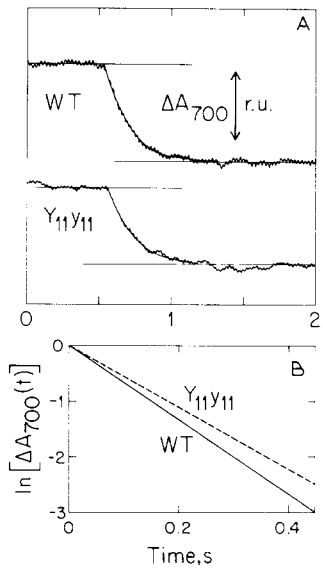

Fig. 3. (A) Absorbance change kinetics of P700 (ΔA_{700}) upon illumination of wild type (WT) and $Y_{11}y_{11}$ thylakoids. The reaction mixture contained either 170 μM Chl (WT) or 150 μM Chl ($Y_{11}y_{11}$) and 20 μM DCMU with 240 μM methyl viologen. The actinic light came on at 0.55 s. The optical pathlength of the cuvette was 0.18 cm. (B) Semilogarithmic plot of the absorbance change kinetics. The slope of each line defined the rate constant of light absorption by PS I (K_I) in wild-type ($K_I = 6.4\ s^{-1}$) and $Y_{11}y_{11}$ ($K_I = 5.3\ s^{-1}$) thylakoids.

Chl/Q_A ratios. Similar reaction center enrichment was reported for the Chl-*b*-less chlorina f2 mutant of barley [13]. Fig. 2B shows the stoichiometric ratio of PS II and PS I reaction centers in the three soybean strains: the PS II/PS I ratio increases as a function of the Chl *a*/Chl *b* ratio, i.e., from a value of about 1.9 in the wild-type (Chl *a*/Chl *b* = 3.2) to PS II/PS I = 3.5 when the Chl *a*/Chl *b* ratio approaches 9.0.

Since all Chl *b* in the thylakoid membrane of chloroplasts is associated with the LHC of PS II and of PS I, it is expected that a Chl *b* deficiency will affect the functional antenna size of both photosystems. We tested for this expectation by comparing the functional chlorophyll antenna size of the two photosystems in the various samples. This was implemented by measuring the rate of light absorption by each photosystem under broad green actinic excitation, using thylakoids from plants with different Chl *a*/Chl *b* ratios [20]. The rationale for this approach is that for each photosystem, the rate of light absorption is directly proportional to the chlorophyll antenna size. For illustration purposes, we present kinetic data from wild-type and $Y_{11}y_{11}$ samples only. Fig. 3A shows the light-induced absorbance change kinetics at 700 nm (ΔA_{700}) with wild-type (Chl *a*/Chl *b* ratio of 3.4) and greenhouse-grown 17-day-old $Y_{11}y_{11}$ (Chl *a*/Chl *b* ratio of 5.5) chloroplasts. Fig. 3B compares the semilogarithmic plot of the above kinetic traces. The rate of trap closure, hence the rate of light absorption by PS I (K_I) was measured directly from the slope of the semilogarithmic plots. We determined K_I (wild-type) $= 6.4\ s^{-1}$ and K_I ($Y_{11}y_{11}$) $= 5.3\ s^{-1}$, suggesting a smaller PS I photosynthetic unit size for the mutant compared to wild type.

The rate of light absorption by PS II was measured from the kinetics of fluorescence induction in the presence of DCMU [20]. Fig. 4A shows fluorescence induction traces of dark-adapted DCMU-treated wild-type and Clark $Y_{11}y_{11}$ thylakoids. The kinetic analysis of the area over the fluorescence induction curves is presented in Fig. 4B. The semilogarithmic plot of the growth of the area over each fluorescence induction curve reflects the reduction kinetics of the PS II primary

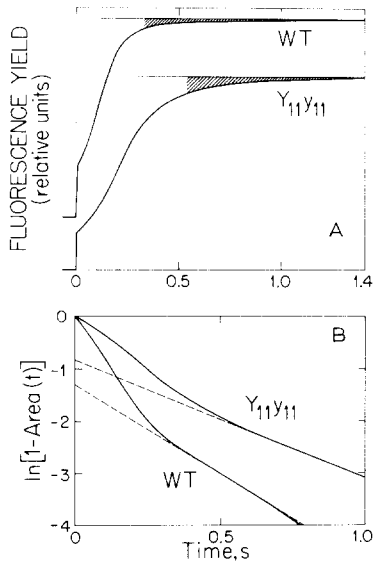

Fig. 4. (A) Fluorescence induction kinetics of DCMU-treated wild-type (WT) and $Y_{11}y_{11}$ thylakoids. The reaction mixture contained either 120 μM Chl (WT) or 100 μM Chl ($Y_{11}y_{11}$), 20 μM DCMU and 200 μM FeCN. The shaded areas mark the portion of the fluorescence induction trace where the activity of PS II_β is the only component remaining in the kinetics. (B) Semilogarithmic plot of the growth of the area over the fluorescence induction curve (solid line). Biphasic kinetics reflect the contribution of a slow, exponential component (PS II_β) and of a faster sigmoidal component (PS II_α). The rate constant of the slow component, K_β, was determined from the slope of the slow linear phase. The relative amount of PS II_β centers was determined from the y-axis intercept of the slow linear phase (dashed line).

quinone acceptor Q_A to Q_A^- [5]. All thylakoid membrane samples displayed biphasic kinetics, suggesting heterogeneity in the organization of PS II. The contribution of the slow exponential component was estimated by extrapolating the slow linear phase of each semilogarithmic trace in Fig. 4 (lower) to the y-axis. The anti-ln of the intercept with the y-axis provided a direct measure of the fraction of PS II complexes with smaller absorption cross-section. This fraction of PS II complexes was estimated to be equal to 23% and 50% of the total PS II in wild-type and $Y_{11}y_{11}$ samples, respectively. The substantial difference in the amount of PS II_β between wild-type (WT) and mutant ($Y_{11}y_{11}$) is seen qualitatively in Fig. 4 (upper) from the shaded area over the respective fluorescence induction curves. The rate of light absorption by the slow photoactivity centers (K_β) was estimated directly from the slope of the straight line in the semilogarithmic plots as follows: K_β (wild type) = 3.5 s^{-1} and K_β ($Y_{11}y_{11}$) = 2.3 s^{-1}. The kinetics of the fast component were resolved upon deconvolution of the biphasic phenomenon, i.e., upon subtraction of the slow phase from the overall kinetic phenomenon [5]. The corresponding rates of light absorption (K_α) were estimated from each fast component, K_α (wild-type) = 7.4 s^{-1} and K_α ($Y_{11}y_{11}$) = 4.2 s^{-1}, suggesting substantial reduction in the antenna size of PS II_α in the mutant plant.

From the relative photosystem concentration (Chl/P700, Chl/Q_A) and from the rate of light absorption by each photosystem, we were able to calculate the number, N, of chlorophyll molecules that are functionally associated with each photosystem [20,23]. Table I summarizes the results. Thylakoids from wild-type soybean plants have a reaction center stoichiometry of about 1.9, similar to those reported for other higher plant chloroplasts [24]. The functional chlorophyll antenna size of the photosystems in wild-type soybean chloroplasts (N_I = 210, N_α = 250 and N_β = 115) agrees with values reported for spinach [23], pea [25], maize [20] and barley [13] chloroplasts.

The Clark y_9y_9 mutant showed an elevated PS II/PS I stoichiometry and a higher relative amount of PS II_β centers. Comparison of the relative concentration of PS II_α, PS II_β and PS I in wild-type and y_9y_9 thylakoids revealed the following ratios: wild-type, $\alpha/\beta/I = 1.5/0.4/1.0$; y_9y_9, $\alpha/\beta/I = 1.6/0.9/1.0$. These numbers suggest that a higher PS II/PS I stoichiometry in this mutant could be largely accounted for as an increase in the relative concentration of PS II_β (Table I). The chlorophyll antenna sizes of PS II_α and PS I are smaller than those of wild-type plants. The antenna size of PS II_α was lowered from 250 Chl $a+b$ molecules in the wild type to about 150 Chl $a+b$ molecules in the y_9y_9, a reduction of 40%. Similarly, the antenna of PS I was lowered from 210 to 160 Chl $a+b$ molecules, a reduction of 24% (Table I). In contrast, the antenna size of PS II_β remained unchanged, suggesting that a primary chloroplast response under conditions of limited Chl b availability is to eliminate the assembly of the peripheral component of LHC II and most of

TABLE I

PHOTOCHEMICAL APPARATUS CHARACTERISTICS OF SOYBEAN WILD-TYPE AND CHL-*b*-DEFICIENT MUTANT CHLOROPLASTS

The relative concentration of Chl, P700, Q_A is given on a mol : mol basis. The concentration of PS II_β is given as percent of the total PS II in the thylakoid membrane. The ratio of PS II_α/PS II_β/PS I (α/β/I) is based on the relative concentration of PS I (P700). The number of the functional chlorophyll molecules associated with PS I, PS II_α and PS II_β is given by N_I, N_α and N_β, respectively.

	Wild-type	y_9y_9	$Y_{11}y_{11}$
Chl *a*/Chl *b*	3.4±0.1	5.0±0.1	5.5±0.2
Chl/P700	650±50	500±15	480±50
Chl/Q_A	340±50	200±25	170±30
PS II/PS I	1.9±0.2	2.5±0.2	2.8±0.3
[PS II_β], %	23±9	37±10	43±7
α/β/I	1.5/0.4/1.0	1.6/0.9/1.0	1.6/1.2/1.0
N_I	210	160	170
N_α	250	150	140
N_β	115	110	75

the LHC I [2]. However, the assembly of the tightly bound LHC II (LHC II-inner), which is present in PS II_β, is not affected by the y_9y_9 mutation. It is important to observe that both the relative concentration of PS II_β and the chlorophyll antenna size of PS II_α and PS I reverted to those of wild type upon completion of the development of the mutant (not shown).

The Chl *b* deficiency in the Clark $Y_{11}y_{11}$ mutant is more severe than in the y_9y_9 mutant. Accordingly, thylakoids from the $Y_{11}y_{11}$ show even higher PS II/PS I stoichiometry. Once again, the elevated PS II/PS I ratio in this mutant could be accounted for by the much greater fraction of PS II_β present in the thylakoid membrane (Table I). The antenna size of PS I in the $Y_{11}y_{11}$ contained about 170 Chl $a+b$ molecules, i.e., is about the same as in the less-deficient y_9y_9 mutant. Interestingly, the chlorophyll antenna size of PS II_α was the lowest recorded, i.e., 140 Chl $a+b$ molecules, which is only slightly larger than that of PS II_β in the wild-type. Moreover, the chlorophyll antenna size of PS II_β was also lowered from 110 to about 75 Chl $a+b$ molecules (Table I).

Discussion

In the present work, we addressed the question of photochemical apparatus organization in the Chl-*b*-deficient soybean mutants, the Clark y_9y_9 and Clark $Y_{11}y_{11}$, where the limited amounts of Chl *b* present resulted in a chloroplast developmental condition intermediate to those of wild-type and the Chl-*b*-less chlorina f2 mutant of barley [13]. We found three distinct effects on the organization and function of the photosystems [26]: (a) Smaller chlorophyll antenna size of the photosystems; (b) higher PS II/PS I reaction center stoichiometry; (c) enhanced PS II_β content (Table I).

The Chl *b* deficiency in the two soybean mutants resulted in smaller functional antenna size for the photosystems, suggesting that both mutations involve the biosynthesis and/or assembly of the LHC I and LHC II. The most pronounced change occurred in the chlorophyll antenna size of PS II_α, which was lowered from about 250 Chl $a+b$ molecules in the wild type, to about 150 Chl $a+b$ molecules in the y_9y_9, and to about 140 Chl molecules in the $Y_{11}y_{11}$. Thus, the mutations caused the loss of 40–50% of the chlorophyll antenna of PS II_α whereas, by comparison, the loss of chlorophyll from the antenna of PS I was only about 20% (Table I).

Concomitant with reductions in chlorophyll antenna size, the Chl-*b*-deficient mutants displayed enhanced PS II/PS I stoichiometries. The enhancement in the ratio of photosystem stoichiometry correlated with the degree of Chl *b* deficiency, suggesting a cause-and-effect relationship: since the chlorophyll antenna size of PS II_α was lowered by about 50%, whereas that of PS I was lowered by 20% only, it appears that light absorption by PS II was attenuated more than that of PS I. In turn, an elevated PSII/PSI ratio might be a response of the plant in establishing a balanced absorption of light by the two photosystems. Our analysis revealed that in all cases, a higher PS II/PS I stoichiometry could be accounted for by the enhanced relative concentration of PS II_β. Such response by the chloroplast is meaningful given the Chl *b* deficiency on the one hand and the low Chl *b* content of PS II_β on the other. However, a distinction must be made between PS

II_β in the wild-type and that found in mutant thylakoids.

The definition of PS II_β is based solely on the functional antenna size, which is about 120 Chl $a + b$ molecules for this photosystem. In the wild-type chloroplasts, the small pool of PS II_β (PS II_β/PS I = 0.4 : 1.0) presents an aberration in the Q_A–Q_B interaction, as it apparently lacks the gate of two-electron mechanism [8,9] and displays a slow turnover of electrons from the reducing side [10]. Following the nomenclature by Lavergne [9], PS II_β in wild-type chloroplasts is termed PS II_β-non-Q_B. The photochemical activity of PS II_β-non-Q_B is readily reflected in the initial fluorescence yield increase (F_o to F_{pl}) observed either in vivo or with thylakoid membranes suspended in the presence of the artificial electron acceptor potassium ferricyanide [10]. The amplitude of the initial fluorescence yield increase from F_o to F_{pl} provides a measure of the amount of PS II_β-non-Q_B in chloroplasts [10]. Utilizing this property, it was determined that in the Chl-*b*-deficient mutants, the fraction of PS II_β-non-Q_B remained constant (results not shown), suggesting that a greatly enhanced PS II_β content (Table I) comes about because of the addition of PS II_β-Q_B type. The results presented in this work support the notion that PS II_β-Q_B appears exclusively in the mutant chloroplasts under conditions of Chl *b* deficiency and that it participates efficiently in the process of electron transport to the plastoquinone pool. Since under conditions of limited Chl *b* biosynthesis the peripheral complement of LHC II is not assembled and PS II_β accumulates in the thylakoid lamella, it may be concluded that PS II_β is a stable functional entity and may represent an intermediate in the assembly of the PS II light-harvesting antenna.

In an earlier study, we investigated the photochemical apparatus organization of the Chl-*b*-less chlorina f2 mutant of barley [13]. Chloroplasts from the chlorina f2 mutant plants displayed similar changes in the organization of PS II. The light-harvesting antenna of PS II was lowered from about 250 Chl $a + b$ in the wild type to only 50 Chl *a* molecules in the mutant. The PS II/PS I complex ratio was substantially higher, i.e., about 3.0 in the mutant versus about 1.9 in the wild type. In addition, mutant chloroplasts lacked both PS II_α and PS II_β from the thylakoid membrane, since the PS II complexes present possessed a limited antenna of only 50 Chl *a* molecules.

The combined results from the work with the Chl-*b*-less chlorina f2 mutant of barley and from the present work with the soybean y_9y_9 and $Y_{11}y_{11}$ mutants provide information on the interplay between Chl *b* deficiency, PS II antenna size and PS II heterogeneity. They point to a two-step mechanism for the development, assembly and organization of the photosynthetic unit of PS II [13]:

$$\text{PS II}\ (50\ \text{Chl}\ a) \xrightarrow{\text{LHC II-inner}} \text{PS II}_\beta\ (120\ \text{Chl}\ a+b) \xrightarrow{\text{LHC II-peripheral}} \text{PS II}_\alpha\ (250\ \text{Chl}\ a+b)$$

The above model, however, may not fully account for the observation that the relative concentration of PS II_β-non-Q_B remained constant in the thylakoid membrane and independent of the extent of mutation or developmental stage of the chloroplast. Therefore, while the functional role of PS II_β-Q_B in the mutant chloroplast is understood clearly to be the accumulated precursor of PS II_α and a result of the Chl *b* deficiency, the origin and physiological role of PS II_β-non-Q_B remain to be elucidated.

Acknowledgements

The work was supported by USDA 85-CRCR-1-1577 Competitive Research Grant. We thank Dr. Ken Eskins for providing the soybean mutant seeds.

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