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Photochemical apparatus organization in the thylakoid membrane of *Hordeum vulgare* wild type and chlorophyll *b*-less chlorina f2 mutant

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The stoichiometry of the electron-transport complexes and the chlorophyll (Chl) antenna size of the photosystems is correlated in chloroplasts from barley wild type and in the chlorophyll *b*-less chlorina f2 mutant. Chloroplasts from mutant plants displayed three characteristic changes in the organization of Photosystem I (PS I) and Photosystem II (PS II). (a) 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 light-harvesting antenna of PS I was lowered from about 185 Chl *a* + *b* in the wild type to about 150 Chl *a* molecules in the mutant. (b) The PS II/PS I complex ratio was substantially higher, i.e., about 3.0, in the mutant vs. about 1.8 in the wild type. (c) Mutant chloroplasts lacked the differentiation of PS II into PS II_α and PS II_β. The enhanced PS II/PS I ratio is explained as a response of the plant to the lowered light-harvesting capacity of PS II in the mutant chloroplasts. The apparent lack of PS II heterogeneity in the thylakoid membrane of the chlorina f2 correlates with the absence of the Chl *a*/*b* light-harvesting complex II (LHC II) (Percival, M.P., Webber, A.N. and Baker, N.R. (1984) Biochim. Biophys. Acta 767, 582–589) and suggests a difference in the amount of LHC II associated with PS II_α and PS II_β.

Introduction

Recent work on the structural and functional organization of higher plant chloroplasts suggested a highly adaptive thylakoid membrane in which both the stoichiometry of complexes and the antenna Chl are adjusted and optimized in response to the prevailing environmental conditions [1–5]. It was proposed that a class of such responses is triggered by changes in the balance of electron transport between PS II, the Cyt b_6-*f* and PS I complexes in the thylakoid membrane [6]. In the past, work from this laboratory examined the response of higher plant chloroplasts to changes in the light-quality during plant growth [2,5–7]. It was demonstrated that imbalance in the absorption of light between PS II and PS I and, therefore, imbalance in the rate of electron transport between the two photosystems, triggers a chloroplast response in the form of increased biosynthetic/assembly activity toward the photosystem that presents the rate-limiting step [6]. Thus, changes in the stoichiometry between the two

Abbreviations: Chl, chlorophyll; PS, Photosystem; Q, primary quinone-acceptor of PS II; P-700, reaction center of PS I; Cyt, cytochrome; LHC, light-harvesting complex; FeCN, potassium ferricyanide; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Correspondence address: Dr. Anastasios Melis, Division of Molecular Plant Biology, 313 Hilgard Hall, University of California, Berkeley, CA 94720, U.S.A.

photosystems occur in response to changing light-quality during plant growth.

In addition to variations in the light-quality and light-intensity environments during plant growth, the balance of electron transport between the three main integral complexes in the thylakoid membrane (PS II, Cyt b_6-f and PS I) could be altered by specific mutations. Of particular interest are mutations which affect the biosynthesis and/or assembly of the LHC II. Such mutations attenuate the light-harvesting capacity of PS II [6], and create an electron transport imbalance in favor of PS I. In the present work we addressed the question of chloroplast response to a mutation inhibiting the biosynthesis of Chl *b*. The Chl *b*-less chlorina f2 barley mutant of *Hordeum vulgare* (barley) was first identified and characterized by Highkin and co-workers [8–10]. The mutation consists of a defect in the Chl *b* biosynthetic pathway which results in pale-green plants. The total absence of Chl *b* apparently affects the stability of light-harvesting complex polypeptides in the 25–30 and 20–25 kDa region, respectively, but does not change the levels of their respective mRNAs [11]. Chlorina f2 mutant chloroplasts contain grana although the proportion of the appressed membrane area is substantially reduced as compared with their wild-type counterparts [12,13].

In the present work we correlated the structural and functional organization of the electron transport complexes in the wild type and chlorina f2 chloroplasts of *Hordeum vulgare*. When compared to the wild type, the mutant showed a substantially lower PS II light-harvesting Chl antenna size. A smaller change in the Chl antenna size of PS I was also detected. Mutant chloroplasts lacked the differentiation of PS II into PS II_α and PS II_β. The apparent absence of PS II heterogeneity from the thylakoid membrane of the chlorina f2 suggests the involvement of the LHC II in this heterogeneity [14]. Mutant chloroplasts also showed a marked increase in the PS II/PS I stoichiometric ratio. The enhanced PS II/PS I ratio is interpreted as a response of the plant to the lowered light-harvesting capacity of PS II in mutant chloroplasts. It works in the direction of countering the effect of the mutation and reestablishing an overall balanced absorption of light between the two photosystems.

Materials and Methods

Wild-type barley (*Hordeum vulgare*, variety Mariout) and chlorina f2 mutant plants were grown in the greenhouse under controlled conditions. Thylakoid membranes were isolated as previously described [15]. Chlorophyll concentrations were determined in 80% acetone. We used Arnon's equations [16] for the determination of Chl *a* and Chl *b* in wild-type chloroplast samples. The Chl *a* concentration in the chlorina f2 chloroplast samples was estimated from the absorbance at 663 nm using an extinction coefficient of 84 $mM^{-1} \cdot cm^{-1}$ [17].

The concentration of PS II and PS I reaction center complexes was determined from the amplitude of the light-minus-dark absorbance changes at 320 nm and at 700 nm, respectively [18]. The optical pathlength of the cuvette was 0.2 cm. The differential flattening correction factors at 320 nm [19] were about 1.3 for wild-type chloroplasts and 1.15 for the chlorina f2 mutant. The concentration of the Cyt b_6-f complex was determined from the reduced-minus-oxidized absorbance difference spectrum of Triton-solubilized chloroplasts [20], using an Aminco DW2 spectrophotometer interfaced with a Hewlett-Packard HP 86B computer. The concentration of the photochemically active plastoquinone pool was determined from the light-minus-dark absorbance change at 263 nm [21]. The differential flattening correction factors at 263 nm were about 1.9 for the wild-type chloroplasts and 1.6 for the mutant chloroplasts. The optical pathlength of the cuvette was 0.18 cm and we applied a differential extinction coefficient of 13 $mM^{-1} \cdot cm^{-1}$ in the estimation of the PQ concentration.

The functional Chl antenna size of PS II and PS I was estimated directly from the rate of light absorption by each photosystem [15,18]. Actinic excitation of uniform field was provided by broad-band green light transmitted by a combination of CS 4-96 and CS 3-68 Corning filters. The light intensity was set at 30 $\mu mol\ m^{-2} \cdot s^{-1}$ for all kinetic measurements described.

The rate of light absorption by PS I (K_I) was determined from the rate of P-700 photooxidation (ΔA_{700}) in KCN-treated chloroplasts. The rate of light absorption by PS II_α and PS II_β (K_α, K_β)

was determined from the rate of Q photoreduction (ΔA_{320}) and from the fluorescence induction kinetics in DCMU-poisoned chloroplasts. Signal averaging and kinetic analyses were performed by an on-line Hewlett-Packard HP86B computer interfaced with a high-speed voltmeter.

Chloroplast fractionation was implemented upon passing isolated chloroplasts twice through a Yeda press at 16.35 MPa pressure. Grana thylakoids were collected by centrifugation at 40 000 × *g* for 30 min. A light membrane fraction containing mostly intergrana lamellae was collected from the supernatant by further centrifugation at 100 000 × *g* for 1 h. Resolved membranes from the grana partition regions (BBY particles *) were isolated as described previously [22].

Results

Quantitation of electron-transport complexes

Wild type barley chloroplasts have Chl *a*/Chl *b* ratios in the range of 2.8–3.0, characteristic of chloroplasts with extensive regions of stacked membranes. The chlorina f2 barley mutant completely lacked Chl *b*. The concentration of PS I, Cyt b_6-*f* and PS II complexes in isolated chloroplasts of wild type and mutant is shown in Table I. The amount of photoreducible plastoquinone was estimated directly from the light-induced absorbance difference signal at 263 nm [21]. Fig. 1 shows the kinetics of the absorbance change at 263 nm, attributed to plastohydroquinone formation in wild type (WT) and mutant (M) chloroplasts. Table I summarizes the quantitation of electron-transport component data from the two chloroplast samples. A reduction of Chl on a per-component basis is evident in the mutant chloroplasts, in agreement with Thornber and Highkin [10], reflecting the absence of the Chl *a*/*b* LHC. The stoichiometry ratio of electron-transport components were Q/PQ/Cyt *f*/P-700 = 1.8 : 9.4 : 1.1 : 1.0 in the wild type and Q/PQ/Cyt *f*/P-700 = 3.0 : 10.8 : 1.0 : 1.0 in the mutant chloroplasts (Table I). Thus, the relative concentrations

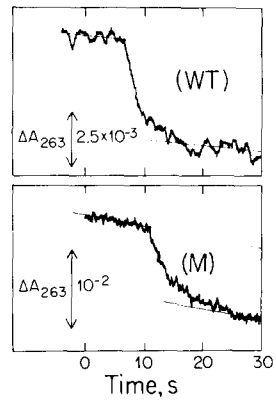

Fig. 1. Time-course of plastoquinone photoreduction in barley wild type (upper trace, 210 μM Chl), and chlorina f2 mutant (lower trace, 208 μM Chl) chloroplasts. The actinic light was turned on at about 12 s.

of PQ, Cyt b_6-*f* and PS I remained relatively unaffected by the mutation. However, the mutant displayed substantially higher amounts of PS II reaction center complexes relative to the wild type (an increase by a factor of about 1.7, Table I). The mutation reduced the light-harvesting capacity of PS II by preventing the stabilization/assembly of LHC II [11]. Hence, the elevated relative concentration of PS II in the mutant may be thought of as a response of the plant in restoring the balance of light absorption between PS II and PS I (see Discussion).

Determination of light-harvesting chlorophyll antenna size

In order to determine the functional Chl an-

TABLE I

CONCENTRATION OF ELECTRON-TRANSPORT COMPONENTS IN WILD TYPE AND CHLORINA f2 MUTANT BARLEY CHLOROPLASTS

Component ratio	Wild type	Chlorina f2 mutant
Chl *a*/Chl *b*	2.9	∞
Chl/Q	332 ± 26	104 ± 19
Chl/P-700	595 ± 33	313 ± 11
Chl/PQ	63 ± 7	29 ± 6
Chl/Cyt *f*	541 ± 64	325 ± 39
Q/P-700	1.8	3.0
PQ/P-700	9.4	10.8
Cyt *f*/P-700	1.1	1.0

* BBY particles are called after Berthold, Babcock and Yocum (see Ref. 22).

tenna size of each photosystem in barley, we measured the rate of light absorption by each photosystem in the wild type and chlorina f2 mutant chloroplasts. We worked with low-intensity continuous broad-band green actinic light which excites Chl *a* and Chl *b* molecules fairly equally [15]. The rate of light absorption by a pigment bed is directly proportional to the incident light intensity and to the number of light-absorbing molecules. Given that the former parameter is constant in our measurements, any difference in the rates of photochemistry of PS II and PS I must be attributed to differences in the light-harvesting antenna size (i.e., functional chlorophyll/reaction center ratio).

Barley wild-type chloroplasts. The rate of light absorption by PS I (K_I) was determined directly from the rate of P-700 photooxidation in KCN-poisoned chloroplasts. Fig. 2 (upper) shows the kinetics of P-700 photooxidation. A semilogarithmic plot of the kinetics (inset) revealed a monophasic first-order function of time. The slope of the semilogarithmic straight line defined the rate of light absorption by PS I ($K_I = 5.8\ s^{-1}$).

The rate of light absorption by PS II_α (K_α) and by PS II_β (K_β) was determined directly from the rate of Q photoreduction (ΔA_{320}). Fig. 2 (lower) shows the kinetics of the absorbance change at 320 nm of DCMU-poisoned chloroplasts suspended in the presence of potassium ferricyanide. The inset shows a semilogarithmic plot of the ΔA_{320} kinetic trace. Deconvolution of the biphasic PS II kinetics [15,23] defined the kinetic properties of PS II_α (dashed line in Fig. 2, inset) and of PS II_β (linear slow-phase in Fig. 2, inset). The semilogarithmic plots of the activity of PS II_α and PS II_β defined the respective rates of light absorption ($K_\alpha = 8.7\ s^{-1}$, $K_\beta = 5.6\ s^{-1}$). In addition, the concentration of PS II_β was equal to 34% of all PS II reaction centers in wild-type chloroplasts (not shown).

The rate of light absorption by PS II_α and PS II_β was also measured from the Chl fluorescence induction kinetics. Fig. 3 (DCMU) shows the biphasic fluorescence induction kinetics (upper) and a semilogarithmic plot of the area growth (lower). The biphasic kinetics measured in the presence of DCMU reflect the photochemical activity of PS II_α and PS II_β. Analysis of the biphasic kinetics

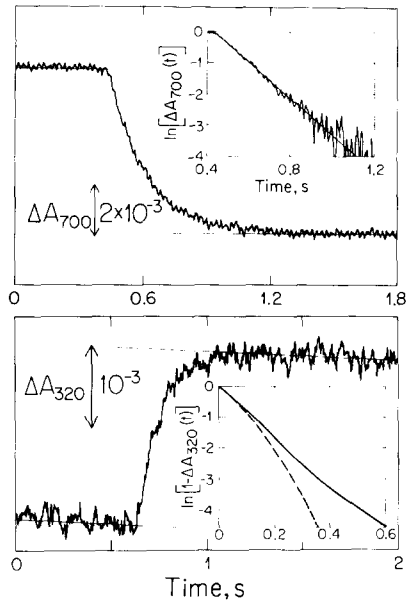

Fig. 2. (Upper) Kinetics of P-700 photooxidation (ΔA_{700}) in wild-type barley chloroplasts. The reaction mixture contained 230 μM Chl, 200 μM methyl viologen, and 20 μM DCMU. The actinic light was turned on at 0.45 s. Inset: semilogarithmic plot of the absorbance change kinetics. The slope of the straight line defined the rate of light absorption by PS I (K_I). (Lower) Kinetics of Q photoreduction (ΔA_{320}) in wild-type barley chloroplasts induced by weak continuous green actinic light. The reaction mixture contained 235 μM Chl, 20 μM DCMU and 800 μM FeCN. The actinic light was turned ON at 0.65 s. The optical pathlength of the cuvette was 0.2 cm. Inset: semilogarithmic plot of the absorbance change kinetics. Zero time corresponds to the time when actinic light was turned on. The biphasic curve reflects the activity of PS II_α and PS II_β.

[15,18,23] defined the rate of light absorption by PS II_α and PS II_β ($K_\alpha = 10\ s^{-1}$ and $K_\beta = 5.6\ s^{-1}$). From this analysis a PS II_β concentration equal to 26% of the total PS II was derived.

It is known that with chloroplasts in the presence of DCMU, determination of K_β requires a very accurate measurement of F_{max}. Very small errors in the measurement of F_{max} would lead to large changes in the value of K_β [24]. To increase confidence in our K_β quantitation, the rate of light absorption by PS II_β was measured, independently, from the initial fluorescence rise kinetics F_0 to F_{p1} [25,26] obtained with chloroplasts in the absence of DCMU, but in the presence of 2 mM potassium ferricyanide. Under these conditions

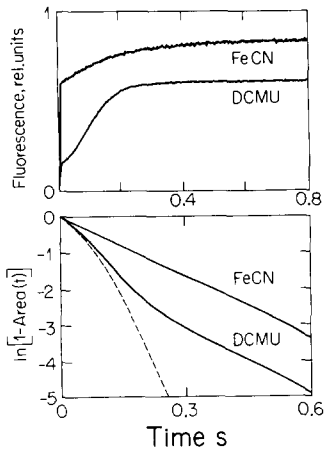

Fig. 3. (Upper) Fluorescence induction kinetics of FeCN-treated or DCMU-treated wild type barley chloroplasts. The reaction mixture contained about 70 μM Chl and either 2 mM FeCN or 20 μM DCMU. The gain of the apparatus for the FeCN trace was set at 4X that of the DCMU trace. Actinic light was turned on at zero time. The maximum fluorescence yield for the DCMU-treated chloroplasts was equal to 0.6 relative units and its was 0.84 relative units for FeCN-treated chloroplasts. (Lower) Semilogarithmic plot of the growth of the area over the fluorescence induction curves. DCMU-treated chloroplasts showed biphasic kinetics, reflecting the activity of PS II_α and PS II_β. The value of K_β was determined from the slope of the slow linear phase. The value of K_α was determined from the initial slope of the dashed line. FeCN-treated chloroplasts showed monophasic kinetics. The slope of the straight line in the FeCN trace defined the rate of light absorption by PS II_β (K_β).

only the kinetic component of PS II_β is expressed [26], thus eliminating the requirement of measuring the value of F_{max}. Fig. 3 (FeCN) shows the monophasic exponential function of PS II_β photoactivity (upper) and the corresponding semilogarithmic plot (lower). In agreement with the DCMU-poisoned sample, the rate of light absorption by PS II_β was defined from the straight line in the semilogarithmic plot ($K_\beta = 5.7\ s^{-1}$). Table II summarizes the relative rates of light absorption by PS I, PS II_α and PS II_β in wild-type barley chloroplasts.

Based on the overall PS II/PS I reaction center ratio of 1.8 (Table I) and the observation that about 30% of the total PS II is PS II_β (Table II), we estimated the following molecular Chl to PS ratios, Chl/PS II_α/PS II_β/PS I = 595 : 1.26 : 0.54 : 1. From these ratios and from the experimentally determined values of the rate of light absorption K_α, K_β and K_I, the absolute numbers N_α, N_β and N_I of Chl molecules transferring excitation to the reaction centers of PS II_α, PS II_β and PS I, respectively, were determined. This was implemented by the solution of the following system of equations [15,18]:

$$\frac{\text{Chl}}{\text{PS I}} = \frac{\text{PS II}_\alpha}{\text{PS I}} N_\alpha + \frac{\text{PS II}_\beta}{\text{PS I}} N_\beta + N_I \quad (1)$$

$$K_\alpha = cIN_\alpha \quad (2)$$

$$K_\beta = cIN_\beta \quad (3)$$

$$K_I = cIN_I \quad (4)$$

where Chl/PS I is the ratio of the total Chl $a + b$ per PS I reaction center, I the actinic light intensity and c a proportionality constant depending on the quantum yield of photochemistry at each photosystem. Although the quantum yield of photochemistry at the three photosystems is not unity, it is generally accepted that it is greater than 0.8 [15,18]. For the solution of Eqns. 1–4 we assumed that the quantum yields of photochemistry at PS II_α, PS II_β and PS I are similar, thereby using the same proportionality constant c. The resulting values of $N_\alpha = 260$, $N_\beta = 170$ and $N_I = 175$ (Table II) are similar to those reported for spinach, pea and maize chloroplasts [7,15,18].

To obtain an independent verification of the functional antenna size of the three photosystems we repeated the above spectrophotometric and kinetic analysis with isolated stroma-exposed lamellae and with resolved membranes from the grana partition regions (BBY particles). Stroma-exposed lamellae contain PS I and PS II_β [27–29] whereas the membrane of the grana partition region contains exclusively PS II_α [29,30].

Stroma lamellae had a Chl a/Chl b ratio of 6.3, Chl/Q = 828 and Chl/P-700 = 234 (see Table II), i.e., a reaction center ratio of PS II/PS I = 0.28 [29]. The kinetic analysis of the absorbance change at 700 nm (ΔA_{700}) defined the rate of light absorption by PS I ($K_I = 5.5\ s^{-1}$, see Table II). Fluorescence induction kinetic analysis of stroma lamellae revealed a single monophasic exponential function of time occurring with rate constant identical to that of PS II_β ($K_\beta = 5.2\ s^{-1}$, see Table II).

TABLE II

PHOTOSYSTEM CONCENTRATION, RATE OF LIGHT ABSORPTION AND CHLOROPHYLL ANTENNA SIZE IN THE THYLAKOID MEMBRANE OF WILD-TYPE BARLEY CHLOROPLASTS

[PS II_β] is given in % of total PS II (Q) present in the thylakoid membrane.

Photochemical parameter	Unfractionated thylakoids	Isolated stroma lamellae	Grana partition regions (BBY particles)
Chl *a*/Chl *b*	2.9	6.3	2.0
Chl/Q	332	828	260
Chl/P-700	595	234	4052
K_I (PS I)(s^{-1})	5.8±0.3	5.5±0.3	–
K_α (PS II_α)(s^{-1})	8.6±1.4	–	–
K_β (PS II_β)(s^{-1})	5.6±1.0	5.2±0.2	–
[PS II_β](%)	30	100	0
N_I (PS I)	175	185	–
N_α (PS II_α)	260	–	250
N_β (PS II_β)	170	175	–

The absence of the dominant PS II_α from the isolated stroma thylakoids simplified the calculation of the functional Chl antenna size of PS I and PS II_β. Using Eqs. 1, 3 and 4 only, we estimated $N_I = 185$ and $N_\beta = 175$ Chl$a + b$ in stroma thylakoids (see Table II). Thus, antenna size estimates for PS I and PS II_β in isolated stroma thylakoids are in good agreement with the corresponding values obtained in unfractionated thylakoids.

Resolved membranes from the grana partition regions (BBY particles) had a Chl *a*/Chl *b* ratio of 2.0, Chl/Q = 260 and Chl/P-700 = 4052 (see Table II). Such preparations were devoid of PS II_β [30] and contained only a small fraction of the total PS I (about 10–15% of that in unfractionated thylakoids). Correcting for the limited PS I contamination in the BBY particles, we estimated a functional antenna size N_α (PS II_α) = 250 Chl$a +$ *b*, in good agreement with that estimated in unfractionated thylakoids (Table II).

Barley chlorina f2 mutant chloroplasts. We provided an evaluation of the functional antenna size of PS I and PS II in the chlorina f2 mutant. Fig. 4 (upper) shows the light-induced kinetics of the absorbance change at 700 nm, attributed to the photooxidation of P-700. The semilogarithmic plot of the kinetics (inset) revealed an exponential function of time and defined a rate of light absorption by PS I, $K_I = 4.5\ s^{-1}$ (Table III). Fig. 4 (lower) shows the light-induced absorbance change

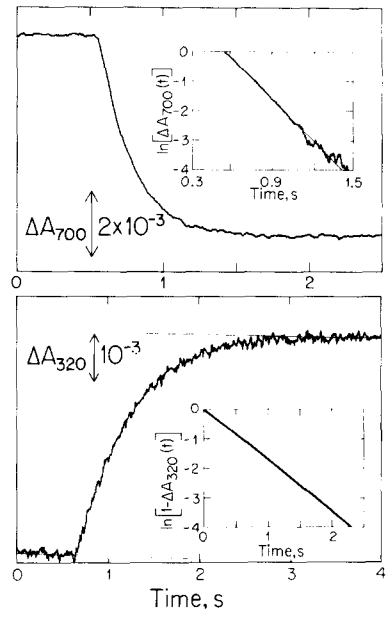

Fig. 4. (Upper) Kinetics of P-700 photooxidation in mutant barley chloroplasts. The reaction mixture contained 174 μM Chl, 200 μM Methyl viologen and 20 μM DCMU. The actinic light was turned on at 0.55 s. Inset: Semilogarithmic analysis of the absorbance change kinetics; the slope of the straight line (K_I) defined the rate of light absorption by PS I. (Lower) Kinetics of Q photoreduction in mutant barley chloroplasts. The reaction mixture contained 205 μM Chl, 20 μM DCMU and 800 μM FeCN. The actinic light was turned ON at 0.65 s. Inset: Semilogarithmic analysis of the absorbance change kinetics. The slope of the straight line (K_{II}) defined the rate of light absorption by PS II in the chlorina f2 chloroplasts.

kinetics at 320 nm, attributed to the accumulation of the semiquinone anion Q^-. In the barley mutant, unlike the wild-type chloroplasts, this process is a single exponential function of time, as already reported by Percival et al. [14]. The semilogarithmic plot of the kinetics (inset) defined the rate of light absorption by PS II in the mutant chloroplasts, $K_{II} = 1.6\ s^{-1}$. A similar estimate of the rate of light absorption by PS II in the mutant chloroplasts was obtained from the measurement of the fluorescence induction kinetics (not shown). Table III summarizes the relative rates of light absorption by PS I and PS II in the barley mutant chloroplast.

Since barley mutant chloroplasts kinetically showed a single type of PS II, the system of Eqns. 1–4 was simplified to:

$$\frac{\text{Chl}}{\text{PS I}} = \frac{\text{PS II}}{\text{PS I}} N_{II} + N_I \quad (5)$$

$$K_{II} = cIN_{II} \quad (6)$$

$$K_I = cIN_I \quad (7)$$

The solution of the above system of equations yielded $N_I = 150$ and $N_{II} = 50$ (see Table III). Thus, the light-harvesting antenna of PS I in mutant chloroplasts is about 20% smaller than that of PS I in wild-type chloroplasts. The uniform antenna size of PS II in the mutant is substantially smaller than that of both PS II_α and PS II_β centers in the wild type.

TABLE III

RATES OF LIGHT ABSORPTION BY PS I (K_I) AND PS II (K_{II}), AND FUNCTIONAL ANTENNA SIZE OF THE PHOTOSYSTEMS IN THE CHLORINA f2 BARLEY MUTANT CHLOROPLASTS

[PS II_β] is given in % of total PS II (Q) present in the thylakoid membrane.

Photochemical parameter	Unfractionated mutant thylakoids
K_I (PS I)(s^{-1})	4.5±0.7
K_{II} (PS II)(s^{-1})	1.6±0.4
[PS II_β](%)	100
N_I (PS I)	150
N_{II} (PS II)	50

Discussion

The inability of the barley chlorina f2 mutant to synthesize Chl *b* had three specific effects in the organization and composition of the photosystems: (a) substantial reduction in the Chl light-harvesting antenna of both photosystems; (b) alteration in the stoichiometry of PS II and PS I reaction centers; and (c) inhibition in the development of PS II heterogeneity.

Light-harvesting antenna in chlorina f2 mutant

The lack of Chl *b* in the chlorina f2 mutant of barley resulted in a PS I functional antenna size reduction from 185 Chl *a* + *b* in the wild type to about 150 Chl *a* in the mutant, i.e., a reduction by about 20% in the number of Chl molecules specifically associated with PS I. Given that chloroplasts of the chlorina f2 mutant totally lack the LHC I [11,31], we suggest that the antenna size of PS I in the mutant chloroplasts provides a close estimate of the size of the core antenna of PS I associated with the P-700-containing 70 kDa polypeptides. This number ($N_I = 150$) is consistent with the PS I Chl antenna size of cyanobacteria ($N_I = 140$ Chl *a*) which is devoid of LHC I and of Chl *b* [3,32].

The absence of Chl *b* from the chlorina f2 chloroplast resulted in a drastic reduction of the PS II functional antenna size from about 250 Chl *a* + *b* in the wild type to about 50 Chl *a* in the mutant, i.e., a reduction by about 80% in the number of Chl molecules specifically associated with PS II_α. In addition, the kinetic differentiation of PS II into PS II_α and PS II_β was no longer evident in the mutant in agreement with recent findings by Percival et al. [14]. By analogy with PS I, we conclude that the core antenna complex of PS II contains at most 50 Chl *a* molecules. Such a conclusion is consistent with estimates of the Chl *a* content in the core antenna complex of PS II in cyanobacteria [3,32], in tobacco mutants [33] and in spinach chloroplasts [18]. It must be noted, however, that our measurements on the functional Chl antenna size of the photosystems do not exclude the possibility of some LHC polypeptides being present in the antenna system of PS II and PS I [11,13,34,35].

Stoichiometry of PS II and PS I complexes

As discussed above, the mutation caused a sub-

stantially greater reduction in the LH antenna size of PS II (a reduction by 80%) as compared to that of PS I (a reduction by 20%). This uneven reduction in the LH antenna of the two photosystems would cause a severe attenuation in the rate of light absorption and, by consequence, in the rate of electron turnover by PS II. The substantially elevated PS II/PS I reaction center ratio in the chlorina f2 (see Table I) could be thought of as a response of the plant designed to compensate for the substantially smaller PS II antenna size in the mutant chloroplasts. Such a response suggests the existence of a chloroplast mechanism capable of detecting and correcting electron-transport imbalance between the photosystems in higher plant chloroplasts [6].

Photosystem II heterogeneity

In the absence of LHC II the functional antenna size of PS II contains at most 50 Chl *a* molecules (core Chl *a* complex). This is apparently the case with intermittent light plastids [7,36], certain tobacco mutants [33] and with the chlorina f2 mutant of barley. It is of interest to observe that evidence in the literature suggests a two-step process in the development of the accessory Chl *a*/*b* light-harvesting antenna of PS II. The first step apparently involves the addition of a tightly bound complement of LHC II containing 70 ± 20 Chl $a + b$ molecules. This PS II configuration is evident in plants grown under high light intensity ($N_{PS\,II} = 130$ Chl, Chl *a*/Chl *b* = 6.7 [37]), in developmental mutants where only limited amounts of LHC II are assembled ($N_{PS\,II} = 130$ Chl, Chl *a*/Chl *b* = ≈ 5 [33,38]), and in PS II_β in several plant species ($N_\beta = 130 \pm 30$ [7,15,18,26,33]). In view of this observation, it is tempting to suggest that the functional differentiation of PS II in PS II_α and PS II_β may reflect a basic heterogeneity and a two-step process in the association of the LHC II with the core complex of PS II. According to this hypothesis, the formation of PS II_β occurs upon addition of a tightly bound complement of LHC (LHC II*) to the PS II-core. The subsequent addition of the peripheral LHC II results in the formation of PS II_α, according to the following scheme:

$$\text{PS II-core} \xrightarrow{\text{LHC II*}} \text{PS } II_\beta \xrightarrow{\text{LHC II}} \text{PS } II_\alpha$$

From the developmental point of view, it may be significant for the chloroplast to synthesize and retain PS II_β in a reservoir form prior to the insertion of the outermost segments of the peripheral LHC II. As this work and the work with other mutants [33,38,39] and chloroplasts in the developing stage shows [7,36] when the supply of the peripheral LHC II is limited, the formation of PS II_α is inhibited and PS II_β accumulates.

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