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Compensatory Alterations in the Photochemical Apparatus of a Photoregulatory, Chlorophyll *b*-Deficient Mutant of Maize[1]

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ABSTRACT

Characterization of the functional organization of the photochemical apparatus in the light sensitive chlorophyll *b*-deficient oil yellow-yellow green (OY-YG) mutant of maize (*Zea mays*) is presented. Spectrophotometric and kinetic analysis revealed substantially lower amounts of the light harvesting complex of photosystem II (LHCII-peripheral) in high light-grown OY-YG thylakoids. However, accumulation of a tightly bound LHCII appears unaffected by the lesion. Changes in photosystem (PS) stoichiometry include lower amounts of PSII with characteristic fast kinetics ($PSII_\alpha$) and a substantial accumulation of PSII centers with characteristic slow kinetics ($PSII_\beta$) in the thylakoid membrane of the OY-YG mutant. Thus, $PSII_\beta$ is the dominant photosystem in the mutant chloroplasts. In contrast to wild type, roughly 80% of the mutant $PSII_\beta$ centers are functionally coupled to the plastoquinone pool and are probably localized in the appressed regions of the thylakoid membrane. These centers, designated $PSII_\beta$-Q_B-reducing (Q_B being the secondary electron quinone acceptor of PSII), are clearly distinct from the typical $PSII_\beta$-Q_B-nonreducing centers found in the stroma lamellae of wild-type chloroplasts. It is concluded that the observed changes in the stoichiometry of electron-transport complexes reflect the existence of a regulatory mechanism for the adjustment of photosystem stoichiometry in chloroplasts designed to correct any imbalance in light absorption by the two photosystems.

The emerging picture of the organization and function of the photosynthetic apparatus in oxygenic photosynthesis is one depicting a dynamic interplay between the stoichiometry of electron transport components and the Chl antenna size of the two photosystems (PSI and PSII), both of which are adjusted and optimized to ensure balanced utilization of light by the two photoreactions (24). This optimization of thylakoid function occurs in response to external (such as light quality and quantity) or internal (developmental and mutational) factors. The present study focuses on a photosynthetic mutant of maize whose unique properties provide novel opportunities for studying the relationship between alterations in the stoichiometric content and antenna size of PSI and PSII.

The dominant OY-YG[2] maize (*Zea mays*) mutant was initially characterized by Hopkins *et al.* (15, 16). A companion paper to this one (14) describes results obtained from the freeze-fracture analysis as well as electrophoretic and immunocytochemical measurements of isolated thylakoids from both the yellow-green heterozygote and the wild-type sibling. Thylakoids from the Chl *b*-deficient OY-YG mutant retained nearly normal levels of the tightly bound LHCII, somewhat reduced quantities of LHCI, and little of the peripheral LHCII antenna. Despite significant limitations in light harvesting Chl antenna size for PSII, growth and development of the yellow-green heterozygotes appeared to be near normal levels. In the present study, we addressed this paradox by investigating the functional organization of the photochemical apparatus in the Chl *b*-deficient mutant. We found that under limiting light conditions, the mutant maintains a high capacity for PSII electron transport, in spite of the limited light-harvesting antenna of individual PSII complexes. This is achieved because of the substantially higher PSII/PSI stoichiometry in the mutant chloroplasts, which compensates for the smaller functional Chl antenna size.

MATERIALS AND METHODS

Plant Growth and Chloroplast Isolation. Maize (*Zea mays*) leaves of both wild type and Chl *b*-deficient mutant OY-YG (15) were harvested from 2 to 3 week old seedlings grown in the greenhouse. The leaves were deveined and ground for 15 s in a Waring blender at 0°C in 50 mM Tricine buffer (pH 7.8), containing 0.4 M sucrose, 10 mM NaCl, and 5 mM $MgCl_2$. The homogenate was filtered through 4 layers of nylon mesh. Chloroplasts were pelleted at 6000*g* for 10 min and resuspended in the same buffer at a Chl concentration of 1 mM. The concentration of Chl and the Chl *a*/Chl *b* ratios were determined in 80% acetone (3).

Spectrophotometric Measurements. Concentrations of P700 and Q_A were determined directly from the amplitude of the light-induced absorbance change at 700 (ΔA_{700}) and 320 nm (ΔA_{320}) (22). Chloroplasts were suspended in the presence of 0.1% SDS, 2 mM sodium ascorbate, and 200 μM methyl viologen for the P700 measurement. The reaction mixture contained 20 μM DCMU and 2.5 mM potassium ferricyanide for the Q_A measurement. We used the procedure of Pulles *et al.* (27) to correct for particle flattening. The average flattening correction factors at 320 nm were 1.37 and 1.25 for wild type and OY-YG, respectively.

Measurements of Cyt *f* were obtained from the room temperature difference spectra of the oxidized (1 mM ferricyanide)

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[2] Abbreviations: OY-YG, oil yellow-yellow green; Q_A, primary electron quinone acceptor of PSII; Q_B, secondary electron quinone acceptor of PSII; LHC, light-harvesting complex; $PSII_\alpha$, PSII with characteristic fast kinetics; $PSII_\beta$, PSII with characteristic slow kinetics; CPII*, LHCII monomer; CPII, oligomeric LHCII; EFs, exoplasmic fracture face of stacked membrane regions; EFu, exoplasmic fracture face of unstacked membrane regions.

minus reduced (3 mM hydroquinone) absorption maximum at 554 nm. We used an Aminco DW-IIa spectrophotometer and defined the peak with respect to the isosbestic points of 543.5 and 560 nm. Reaction mixtures contained 100 μM Chl and 0.15% Triton. The differential extinction coefficient of 18 $mM^{-1}\ cm^{-1}$ (4) was used to calculate Cyt *f* concentrations.

Kinetic measurements, used for determining the rates of light utilization by PSI and PSII, were carried out as described by Melis and Anderson (21). The area over the fluorescence induction curve of DCMU-treated chloroplasts was used to determine the relative concentration and the rate of light-utilization by $PSII_\alpha$ and $PSII_\beta$, whereas the kinetics of the absorbance change at 700 nm were used in determining the rate of light-utilization by PSI. To eliminate secondary electron donation to P700 from upstream donors, thylakoid membranes were incubated with 50 mM KCN to inhibit plastocyanin function (21). For the kinetic measurements, we used green actinic light at an intensity of 50 $\mu E \cdot m^{-2} \cdot s^{-1}$.

To establish the connectivity of PSII centers with the plastoquinone pool, we compared fluorescence induction curves of chloroplasts suspended in the presence and absence of DCMU (20). This correlation allowed us to determine the degree to which both $PSII_\alpha$ and $PSII_\beta$ are functionally linked to the plastoquinone pool and thus to evaluate further the PSII heterogeneity.

RESULTS

Quantitation of Electron-Transport Complexes. Chloroplasts isolated from yellow-green heterozygotes of the dominant OY-YG maize mutant and from wild-type siblings had Chl *a*/Chl *b* ratios averaging 5.6 and 3.6, respectively (Table I). The deficiency of Chl *b* in mutant chloroplasts is evidenced by the reduction in room temperature absorption maxima at 474 and 650 nm (Fig. 1). The two spectra were arbitrarily normalized at 680 nm. A significant difference between the two spectra (Fig. 1) is the supressed Soret band of the wild type which is explained by the relatively stronger particle flattening in these chloroplasts. This result, along with the flattening correction factors at 320 nm (see "Materials and Methods"), suggests that mutant chloroplasts have overall lower pigment density. Such absorption differences are typical for Chl *b*-deficient mutants (26). Total leaf Chl content, measured on a fresh weight basis, was approximately 50% lower for mutant as compared to wild-type plants.

Quantitative estimates of P700 and Q_A content were obtained from the light minus dark difference signal at 700 and 320 nm, respectively (21, 22). Cyt *f* content was estimated from the reduced minus oxidized difference signal at 554 nm. A summary of electron transport component quantitation also appears in Table I. For wild-type chloroplasts, these results are in good agreement with values obtained previously (9).

The most notable phenotypic alteration of the OY-YG chloroplasts was the considerable enrichment of PSII. Since bundle sheath chloroplasts are depleted in PSII (PSII/PSI ratio typically <0.25; [9]) this effect is clearly not accounted for by an enriched bundle sheath contribution in the mutant. Thus, enhancement in PSII concentration, presumably in response to the OY-YG lesion, results in a near doubling of the PSII/PSI ratio shown for the mutant in Table I.

Relative Absorption Cross-Section of PSI and PSII in Wild-Type and Mutant Chloroplasts. Estimates of the functional antenna size of the photosystems in mutant and wild-type chloroplasts were obtained from the rate of light utilization by PSII and PSI under limiting excitation conditions. Figure 2 (upper) shows fluorescence induction traces of DCMU-poisoned chloroplasts from wild-type (WT) and mutant (OY-YG) thylakoids. As the same incident light intensity was used in both measurements, the significantly slower rate of variable fluorescence increase in the OY-YG chloroplasts would be attributed to a smaller PSII antenna size or to a much smaller quantum yield of PSII primary photochemistry in this mutant. Figure 2 (upper) also shows practically identical variable-to-maximal fluorescence ratio (Fv/Fm) in the mutant *versus* the wild-type control, indicating a similar overall quantum yield of primary PSII photochemistry (17, 30). It is implied that different rates of variable fluorescence increase between mutant and wild type probably reflect differences in the Chl antenna size of PSII between the two samples (30).

In Figure 2 (lower), semilogarithmic plots of the area over the fluorescence induction curves defined the rate of light utilization by PSII. The rate constant for $PSII_\beta$ (K_β) is measured from the slow, linear phase and is directly proportional to the functional Chl antenna size of this photosystem. Values for K_β were similar in wild-type and OY-YG samples (4.6 ± 0.4 and 5.1 ± 0.4 photons per s, respectively), indicating that $PSII_\beta$ has about the same Chl antenna size in the two samples. The rate constant K_α of the rate of light-utilization by $PSII_\alpha$ was estimated from the initial slope of the sigmoidal fast phase of the semilogarithmic plot after subtracting the component attributable to $PSII_\beta$ (21). The experimentally determined wild-type value of K_α was 1.4 times that obtained for the OY-YG mutant (10.6 ± 1 s^{-1} and 7.5 ± 0.7 s^{-1}, respectively), indicating a considerably smaller absorption cross section for $PSII_\alpha$ in the mutant *versus* that of the wild type. The intercept of the slow linear phase in Figure 2 (lower) with the ordinate at zero time provided a measure of the relative proportion of PSII centers exhibiting slow $PSII_\beta$ kinetics (23). In this respect, we observed a significant enrichment of $PSII_\beta$-like centers in the mutant (up to 67% of the total PSII) as compared to the $PSII_\beta$ content in the wild type (22%). It is important to note that this increased abundance of $PSII_\beta$, as with the PSII/PSI ratio, cannot be explained by increased bundle-sheath content in our mutant chloroplast preparations because bundle-sheath chloroplasts have a $PSII_\beta$/PSI ratio less than 0.3 (9). On the contrary, the $PSII_\beta$/PSI ratio in OY-YG samples was 1.57 (Table II).

The light-induced photooxidation kinetics of P700 to its $P700^+$ form were determined with plastocyanin-inhibited (KCN-poisoned) chloroplast preparations. By using the same light-intensity conditions as were used for PSII kinetics, we measured the rate of P700 photooxidation to estimate the rate of light-utilization by PSI. As seen in Figure 3, the rate of $P700^+$ accumulation is more rapid for wild-type (WT) than for mutant (OY-YG) chloroplasts. Quantitative determination of the PSI rate constant (K_{P700}) from semilogarithmic plots (Fig. 4;

Table I. *Photosynthetic Component Stoichiometries in Wild-Type and OY-YG Mutant Chloroplasts of Maize*

The concentrations of P700, Q_A, and Cyt *f* were obtained on a per Chl basis from the amplitude of absorbance change measurements at 700, 320, and 554 nm, respectively (see "Materials and Methods," for details). Note the higher Q_A/P700 and Cyt *f*/P700 ratios in the OY-YG mutant. All values are average measurements (±SD) of a minimum of six independent preparations.

Seedling Type	Chl *a*/Chl *b*	Chl/P700	Chl/Q_A	Chl/Cyt *f*	Q_A/P700	Cyt *f*/P700
WT	3.6 ± 0.1	628 ± 66	493 ± 50	529 ± 66	1.27	1.19
OY-YG	5.6 ± 0.6	537 ± 48	229 ± 51	332 ± 26	2.34	1.62

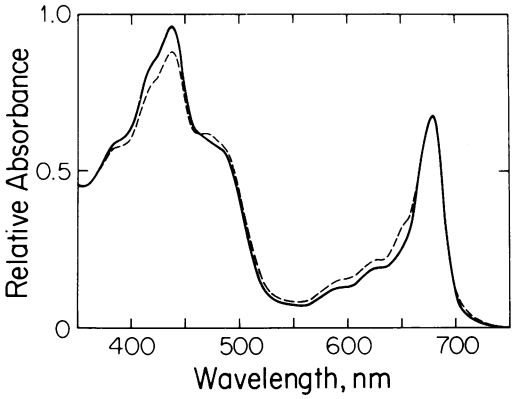

FIG. 1. Room temperature absorbance spectra of wild-type (dashed line) and OY-YG mutant (solid line) isolated chloroplasts. The mutant exhibits relatively reduced absorbance components at 474 and 650 nm, indicating a Chl *b* deficiency.

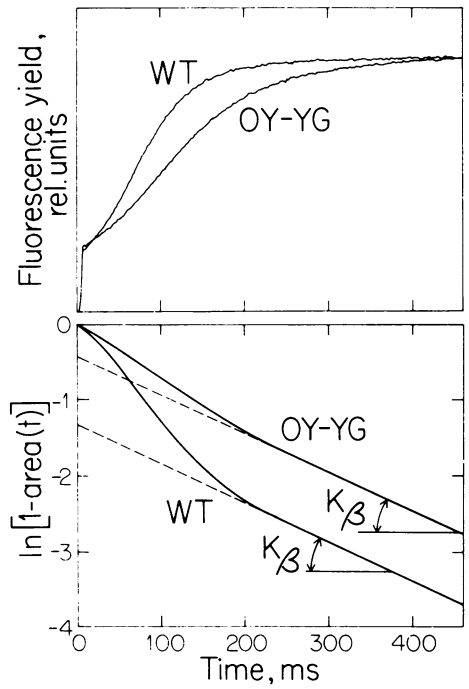

FIG. 2. Upper, Fluorescence induction kinetics of wild-type (WT) and mutant (OY-YG) chloroplasts suspended in the presence of 20 μM DCMU and illuminated with green actinic light (50 $\mu E \cdot m^{-2} \cdot s^{-1}$). Note the relatively slower fluorescence yield increase in the mutant. Lower, Semilogarithmic plots of the area over the fluorescence induction curves for the wild-type (WT) and mutant (OY-YG) chloroplasts. Note the proportionately greater contribution of the slow linear phase in the mutant. Whereas the rate constant for the initial sigmoidal phase (K_α) is smaller for the mutant than for the wild-type samples, the rate constant for the linear component (K_β) is virtually the same.

K_{P700} = 8.4 ± 0.4 s^{-1} and 6.3 ± 0.2 s^{-1} for WT and OY-YG, respectively) provided a direct measure of the rate of light-utilization by PSI in wild type and mutant. Assuming similar quantum yield of PSI photochemistry between mutant and wild type (30), we interpret the kinetic results as reflecting a smaller PSI functional antenna size for the OY-YG phenotype. In Table II, we summarize the kinetics of light-utilization by $PSII_\alpha$, $PSII_\beta$, and PSI. It is observed that significant reductions (by about 30%) in the absorption cross-section of $PSII_\alpha$ and PSI have occurred in the mutant. The kinetics of $PSII_\beta$, on the other hand, remained unchanged in spite of the mutation. These data are consistent with our previous freeze-fracture and electrophoretic analyses (14) and suggest an inherent constancy in $PSII_\beta$ antenna size as opposed to a rather variable Chl content of $PSII_\alpha$ and PSI (10, 12). It is also important to note the substantially altered photosystem stoichiometry in the OY-YG mutant. The enhanced PSII/PSI ratio in the mutant is apparently the result of a fivefold increase in the concentration of $PSII_\beta$ and a simultaneous 30% decrease in the relative concentration of $PSII_\alpha$. Thus, $PSII_\beta$ is the dominant photosystem in the OY-YG mutant, in sharp contrast to the situation prevailing in the wild type (Table II).

Electron Transport Properties of $PSII_\alpha$ and $PSII_\beta$. In addition to a lack of the peripheral complement of LHCII, wild-type $PSII_\beta$ units differ from $PSII_\alpha$ in their lateral distribution in the thylakoid membrane: unlike $PSII_\alpha$, which are localized in the grana partition regions, $PSII_\beta$ have been isolated with a light membrane fraction after Yeda press treatment (2). It was reported (13, 20) that, in mature wild-type chloroplasts, $PSII_\beta$ is not functionally linked to the plastoquinone pool. To investigate whether the above correlations also hold for the abundant $PSII_\beta$ in the OY-YG mutant, we employed fluorescence induction measurements with isolated chloroplasts suspended in the presence of DCMU or in the presence of ferricyanide only. This allowed us to approximate the relative proportion of $PSII_\beta$ centers which are functionally linked with the plastoquinone pool: the initial F_o to F_{pl} fluorescence increase observed for wild-type samples in the presence of ferricyanide normally accounts for about 11% of the total variable fluorescence (Fig. 5). This fluorescence yield increase corresponds to a photoreduction of Q_A in centers that cannot transfer electrons to the plastoquinone pool. The F_o to F_{pl} fluorescence increase displayed β-kinetics and was attributed to the activity of $PSII_\beta$ (20). Thus, the F_o to F_{pl} yield can be used to approximate the fraction of $PSII_\beta$ centers that are not photochemically lined to plastoquinone ($PSII_\beta$-Q_B-nonreducing). Because of the nonlinearity between F_v and O_A^- (25), we estimated approximately 20% of PSII Q_B-nonreducing centers in wild type, *i.e.* in good agreement with the measured $PSII_\beta$ content (22%, Table II). Similar measurements for the OY-YG mutant (not shown) revealed that despite a PSII population predominantly consisting of $PSII_\beta$ (67%, Table II), the amplitude of the F_o to F_{pl} initial fluorescence rise was quite small (about 12% of the total F_v), suggesting that only a small fraction of $PSII_\beta$ (about 20%) in the mutant displays Q_B-nonreducing properties. It may be concluded that unlike the wild type, the majority of $PSII_\beta$ in the mutant are of the Q_B-reducing type.

DISCUSSION

In the current study, the use of spectrophotometric and kinetic analysis allowed us to describe further the modulation of the functional Chl antenna size and photosystem stoichiometry as means by which a Chl *b*-deficient mutant achieves optimal electron flow dynamics. In addition, phenotypic alterations involving contrasting aspects of PSII heterogeneity are discussed.

Effect of Mutation on Photosystem Absorption Cross-Section. The genetic lesion of the OY-YG mutant of maize affects the light-dependent regulation of Chl *b* biosynthesis and/or accumulation. We showed that the OY-YG lesion brings about selective lowering in the concentration of $PSII_\alpha$ relative to PSI. Additional changes occur in the form of smaller PSI light-harvesting antenna (LHCI) and fewer complexes of the peripheral subpopulation of the LHCII. On the contrary, the inner or tightly bound subpopulation of LHCII appears relatively unaffected. From these data we present a model (Fig. 6) summarizing the distribution of Chl-proteins (CP) between $PSII_\alpha$ and $PSII_\beta$ in both the OY-YG and mutant seedlings. We suggest that the peripheral LHCII, corresponding to CPII*/CPII on nondissociating green gels and giving rise to 7 to 9 nm PFs and PFu particles in freeze-fracture electron microscopy, is associated ex-

Table II. *Photoconversion Kinetics of Wild-Type and OY-YG Mutant Maize Chloroplasts*

The rate constants K_α, K_β, and K_{P700} were determined from the photoconversion kinetics of $PSII_\alpha$, $PSII_\beta$, and PSI, respectively, under continuous illumination of limiting intensity. The OY-YG mutant displayed substantially reduced rates of light-harvesting by $PSII_\alpha$ and PSI, whereas the light-utilization kinetics of $PSII_\beta$ were similar to that of the wild type. Additionally, the OY-YG mutant was found to contain a substantial stoichiometric enrichment in $PSII_\beta$ centers and a slight depletion in $PSII_\alpha$ centers.

Seedling Type	K_α	K_β	K_{P700}	%$PSII_\beta$	$PSII_\alpha/PSII_\beta$/PSI
		s^{-1}			
WT	10.6 ± 1	4.6 ± 0.4	8.4 ± 0.4	22 ± 3	0.99/0.28/1
OY-YG	7.5 ± 0.7	5.1 ± 0.4	6.3 ± 0.2	67 ± 5	0.77/1.57/1

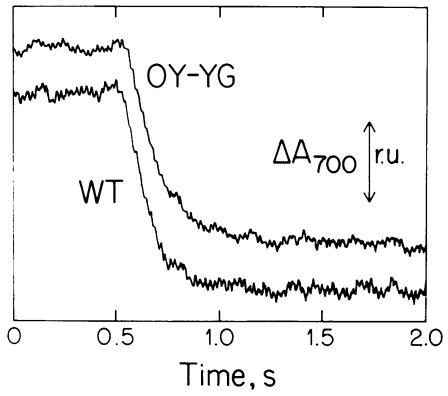

FIG. 3. Light-induced absorbance change kinetics of P700 photooxidation in wild-type (WT) and mutant (OY-YG) thylakoids poisoned by KCN and suspended in the presence of 20 μM DCMU and 200 μM methyl viologen. Actinic light came on at 0.55 s.

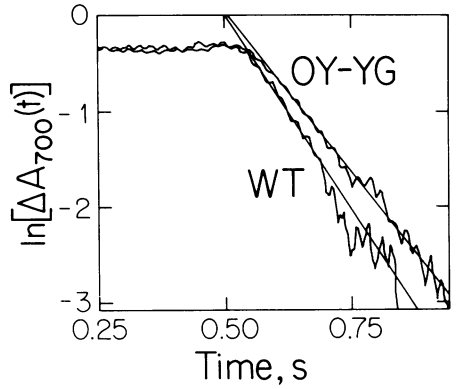

FIG. 4. Semilogarithmic plots of the ΔA_{700} kinetics for the wild-type (WT) and mutant (OY-YG) chloroplasts. Note the slower rate of PSI light-utilization in the mutant.

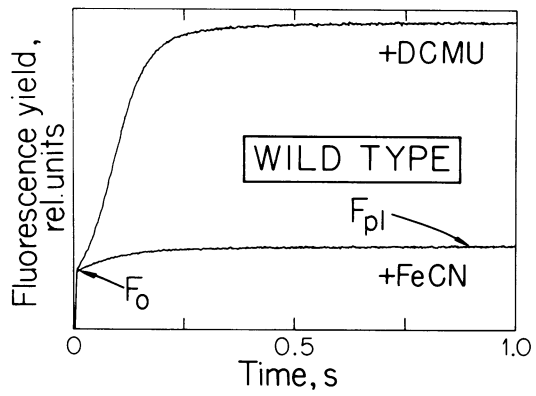

FIG. 5. Fluorescence-induction traces of isolated wild-type membranes suspended in the presence of DCMU (20 μM) or in the presence of 1 mM potassium ferricyanide (FeCN). Note the low relative amplitude of the initial fluorescence rise from F_o to the intermediate plateau F_{pl} (20).

clusively with $PSII_\alpha$. The tightly bound LHCII, partitioning with the PSII core complex as EFs and EFu particles of 8 to 18 nm, is associated with both α and β centers. For reasons of simplicity, all peripheral LHCII units are drawn as identical structures. However, as shown by Dunsmuir (8), the polypeptides associated with CPII*/CPII belong to a multigene family. Some of these possess threonine residues close to their N-terminus that can be phosphorylated during state 1/state 2 transition, while others lack such threonines. Thus, as pointed out by Larsson and Andersson (18), the peripheral LHCII particles probably have a variable composition, and only those possessing phosphorylatable polypeptides would reversely dissociate from $PSII_\alpha$ during state transitions (7, 29). In terms of the OY-YG mutant, a selective reduction in the accumulation of peripheral LHCII subunits results in a subpopulation of PSII centers located in the appressed membrane regions and functionally connected to the plastoquinone pool which, however, display $PSII_\beta$ kinetics of light absorption. These centers, designated $PSII_\beta$ 'Q_B-reducing' ($PSII_\beta$-Q_B) are clearly distinct from the $PSII_\beta$ 'Q_B-nonreducing' ($PSII_\beta$-non-Q_B) centers (6, 19) which are found in stroma lamellae of mature wild-type chloroplasts (20).

The results from this work suggest that the mutation induced differential losses in the Chl *a*/*b* LHC antenna. The light harvesting capacities of both $PSII_\alpha$ and PSI appear substantially reduced in the mutant thylakoids, whereas the efficiency of light-utilization associated with $PSII_\beta$ is unchanged. A decrease of 62% in the relative Chl content of (CPII* + CPII), determined densitometrically from mildly denaturing green gels (14), is paralleled by a reduction of 30% in the overall rate of $PSII_\alpha$ light absorption, supporting our earlier contention (14) that CPII* and CPII represent only the LHCII-peripheral portion of the $PSII_\alpha$ antenna.

Functional Alterations in Photosystem Stoichiometry and PSII Heterogeneity. Our measurements revealed that the yellow-green heterozygotes exhibit a substantially higher PSII/PSI ratio of 2.34 compared to 1.27 in the wild-type siblings, an enrichment which is due to a higher content in $PSII_\beta$ centers. This result is consistent with previous studies of Chl *b*-deficient mutants (1, 11, 24, 26, 28). We suggest that the increased PSII content reflects a compensatory response of the mutant to balance light utilization between the two photosystems and thus overcome the effects of a lesion which acts predominantly to handicap light absorption by $PSII_\alpha$. The relative enrichment in $PSII_\beta$ centers reflects the lack of Chl *b* availability, which prevents the assembly of the peripheral complement of LHCII. The increased PSII/PSI ratio apparent in spectrophotometric studies of the OY-YG mutant is consistent with densitometric analysis of green gels (14) in which

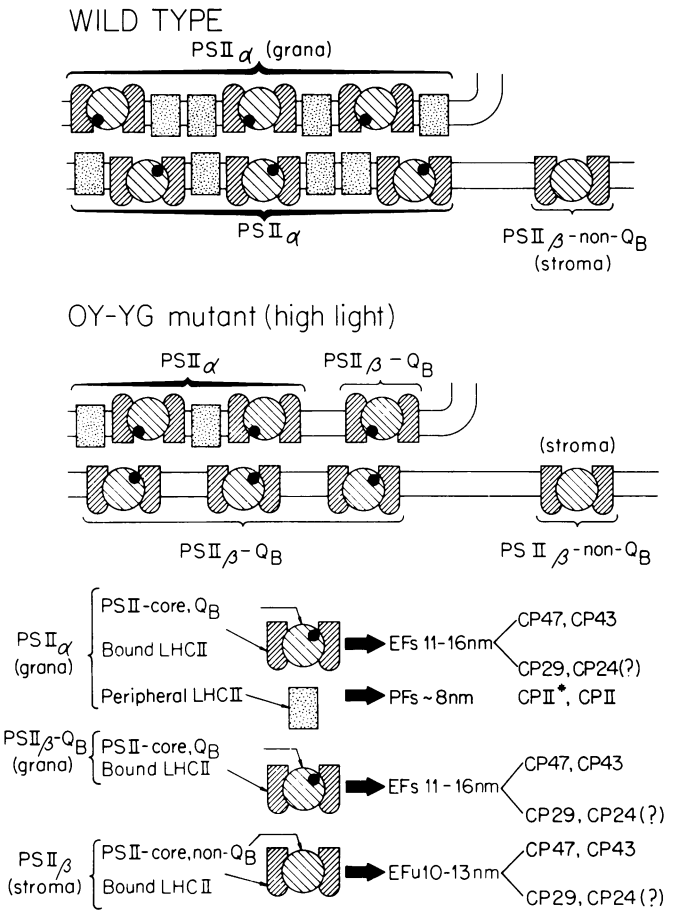

FIG. 6. Schematic diagram of wild-type and OY-YG mutant thylakoid membranes summarizing the main structural and compositional differences of PSII-related complexes in grana and stroma membrane regions. The concept of $PSII_\alpha$ and $PSII_\beta$ centers originated from kinetic spectroscopic data that revealed $PSII_\alpha$-type of complexes to be energetically coupled to each other and $PSII_\beta$ complexes to be separate units. As illustrated in this diagram, it is now possible to relate the spectroscopic 'centers' to structural/biochemical membrane units. Thus, $PSII_\alpha$ centers correspond to PSII complexes located in stacked membrane regions, they display a Q_B-reducing property, and they are surrounded by a pool of LHCII-peripheral complexes that couple the $PSII_\alpha$ units to each other. The $PSII_\beta$ centers that are located in unstacked membrane regions are Q_B-nonreducing (non-Q_B) and derive excitation energy only from inner or tightly bound LHCII antennae. The OY-YG mutation significantly reduces the number of peripheral LHCII complexes in stacked membrane regions compared to the number of PSII complexes with tightly bound LHCII antennae. In the absence of LHCII-peripheral, PSII centers in the grana behave as $PSII_\beta$ in terms of the kinetics of light absorption, even though they still function as Q_B-reducing units and are located in stacked membrane regions.

the ratio of (CP47 + CP43)/(CPI* + CPI), *i.e.* the ratio of PSII-core to the sum of PSI (P700 with and without LHCI), was nearly threefold higher in the mutant as compared to wild-type thylakoids. This figure is somewhat overestimated as the percentage of the Chl associated with PSI (CPI* + CPI) which is contained in LHCI is lower in the mutant. The higher PSII content of the OY-YG mutant was not, however, immediately apparent in the analysis of freeze-fracture micrographs. The ratio of total EF particles (PSII complexes) to PFu particles (representing PSI, Cyt b_6-f, and CF_1-CF_0 complexes [28]) is actually somewhat reduced in the OY-YG mutant, seemingly in contradiction to our measured PSII enrichment. We believe that this discrepancy is due to the relative enrichment of the Cyt b_6-f complex in the stroma-exposed membranes of the mutant and also because of an expected parallel enrichment in ATP synthetase whose CF_0 component appears as 9.5 nm PFu particles, indistinguishable from PSI particles with diminished amounts of bound LHCI. This would be analogous to the enrichment in CF_1 seen in high light-adapted spinach (5).

Evidence from the freeze-fracture work (14) demonstrated that the relative ratio of PSII particles in grana and stroma lamellae (EFs/EFu) for wild-type thylakoids was roughly equal to 4. This is in good agreement with the 80% $PSII_\alpha$ and 20% $PSII_\beta$ distribution found in the wild type, supporting the localization of all $PSII_\beta$ in stroma lamellae. Significantly, this correspondence does not hold for the OY-YG mutant, in which (similarly to wild type) 24% of the EF particles (PSII) were in the unstacked regions, yet from kinetic demonstrations, the mutant has a $PSII_\beta$ content of 67%. It would appear that in the OY-YG mutant only 24% of all PSII is in stroma lamellae, and a substantial number of $PSII_\beta$ complexes in the mutant must then be localized in the membrane of the grana partition region. This conclusion is consistent with the observation that, unlike the wild type, the majority of $PSII_\beta$ in the mutant is functionally connected to the plastoquinone pool. The exact nature of this differential connectivity of $PSII_\beta$ with the plastoquinone pool *versus* membrane localization remains to be elucidated.

Physiological Significance of $PSII_\beta$ with Q_B-Reducing Sites ($PSII_\beta$-Q_B). The enhanced $PSII_\beta$ concentration in mutant chloroplasts is undoubtedly a major factor in explaining why, with such a handicapped light-harvesting ability, the OY-YG mutant sustains overall growth and photosynthesis rates comparable to the wild type. Hence the enhanced $PSII_\beta$ content can be seen as a compensation for the lower light-harvesting capacity in the mutant. Additionally, the higher Cyt *f*/P700 ratio found in the OY-YG mutant, along with a possible enrichment in ATP synthetase, is an indication of a greater electron flux generated by PSII in the mutant relative to the wild type and is similar to changes previously noted for high light-adapted plants (24). An alternative explanation for the increased Cyt *f*/P700 ratio in the OY-YG mutant is to presume that this is truly a high light adaptation resulting from the relatively higher effective light intensity within the mutant leaves due to decreased light absorption and shading, a result of the substantially lower pigment density of the mutant leaves.

The unexplained similarity of high light photoadaptation as it occurs in normal plants to the phenotypic alterations noted for the OY-YG (and other Chl *b* deficient) mutants is a persistent and most intriguing question. The phenotypic alterations are largely comparable, the only apparent difference in the OY-YG response being the extreme degree of sensitivity to changes in light intensity which leads to seemingly 'overadapted' plants under high light conditions (14). We propose that many, if not all of the effects noted for Chl *b* deficiencies can be explained by: (a) intrinsic hierarchical distribution of Chl *b* among the different LHCII polypeptides (14), (b) subsequent rearrangements of the internal pigment bed stoichiometries occurring as a compensatory response to the handicapped light-harvesting abilities of these mutants, and (c) ultrastructural changes of thylakoid stacking based upon internal component changes (most notably, the loss of the peripheral LHCII).

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