Stoichiometries of Photosystem I and Photosystem II in Rice Mutants Differently Deficient in Chlorophyll b

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Stoichiometries of photosystem I (PSI) and photosystem II (PSII) reaction centers in a cultivar of rice, Norin No. 8, and three chlorophyll b-deficient mutants derived from the cultivar were investigated. Quantitation of PSI by photooxidation of P-700 and chromatographic assay of vitamin K₁ showed that, on the basis of chlorophyll, the mutants have higher concentrations of PSI than the wild-type rice. Greater increases were observed in the PSII contents measured by photoreduction of Qₐ, binding of a radioactive herbicide and atomic absorption spectroscopy of Mn. Consequently, the PSII to PSI ratio increased from 1.1–1.3 in the wild-type rice to 1.8 in chlorina 2, which contains no Chi b, and to 2.0–3.3 in chlorina 11 and chlorina 14, which have chlorophyll a/b ratios of 9 and 13, respectively. Measurement of oxygen evolution with saturating single-turnover flashes revealed that, whereas at most 20% of PSI centers are inactive in oxygen evolution in the wild-type rice, the non-functional PSI centers amount to about 50% in the three mutant strains. The fluorescence induction kinetics was also analyzed to estimate proportions of the inactive PSI in the mutants. The data obtained suggest that plants have an ability to adjust the stoichiometry of the two photosystems and the functional organization of PSI in response to the genetically induced deficiency of chlorophyll b.

Key words: Chlorophyll b-deficiency — Mutant — PSI content — PSII content — Photosystem stoichiometry — Rice.

Recently, we have characterized light-harvesting Chl-proteins of Chl b-deficient nuclear gene mutants derived from a cultivar of rice, Norin No. 8 (Terao et al. 1985a, b, 1988, Terao and Katoh 1989). Out of the 16 mutant strains examined, Chi b is totally absent from 10 Type I mutants, chlorina 1 to 10, while three each of the mutants called chlorina 11 to 13 (Type IIA) and chlorina 14 to 16 (Type IIB) have Chl a/b ratios of about 10 and 15, respectively (Terao et al. 1985a). The mutants partially or totally lack LHC-II, a major light-harvesting Chl-protein of PSI, because the apoproteins of LHC-II are normally synthesized but rapidly degraded due to the absence or shortage of Chl b (Terao and Katoh 1989). Contents of LHC-I, an antenna Chl-protein of PSI, are also reduced but in lesser extents than LHC-II in the Type IIA and IIB mutants. Thus, the light-harvesting capacity of PSII is more strongly reduced than that of PSI in the Chl b-deficient mutants. A previous study showed that contents of the intrinsic 47 and 43 kDa Chl proteins of the PSII reaction center complex relative to the 60 kDa Chl proteins of the PSI reaction center complex are significantly larger in the mutants than in the wild-type rice (Terao et al. 1985b). It was suggested that losses of LHC-II are at least partly counterbalanced by increased ratios of PSII to PSI in the mutants (Terao et al. 1988). Similar conclusions have been reached from investigations of Chl b-deficient mutants of other plants, which have higher PSII to PSI ratios than the corresponding wild type plants (Melis and Thielen 1980, Abadia et al. 1985, Ghirardi et al. 1986, Melis and Thielen 1980, Abadia et al. 1985, Ghirardi et al. 1986, Greene et al. 1988).

An elevated PSII to PSI ratio creates, however, another imbalance between the two photosystems. In strong light, electron flow through PSII exceeds that through PSI, resulting in accumulation of electrons in the plastoquinone pool. This in turn would reduce the light-utilization efficiency of photosynthesis and increase susceptibility of PSII to photoinduction. Little attention has so far been paid to this imbalance that should prevail in Chl b-deficient mutants with high PSI to PSII ratios.

In the present study, stoichiometries of PSI and PSII reaction centers in the wild-type and each one of the three types of rice chlorina mutants were investigated. PSI was determined by measuring concentrations of P-700 and vitamin K₁. Five different procedures were used for deter-
mination of PSII because P-680 is difficult to measure by ordinary spectrophotometry and, more importantly, PSII is intrinsically heterogeneous in terms of electron transfer to the plastoquinone pool (Lavergne 1982, Graan and Ort 1986, Chylla et al. 1987, Govindjee 1990). The total PSII was quantified by measuring abundances of pheophytin a, Qa, the herbicide-binding site and Mn, whereas amounts of oxygen evolved per single turnover flash were used to determine the active population of PSII. Relative contents of the active and inactive PSII were also assessed by analyzing the fluorescence induction kinetics. The results were discussed in terms of the plasticity of plants to adjust imbalances in the light absorption and electron transport between PSI and PSII through reorganization of the photosystems.

Materials and Methods

Plant materials—Wild-type rice (Oryza sativa L. cv Norin No. 8) and the three mutant strains derived from the cultivar, i.e., chlorina 2 which completely lacks Chl b and chlorina 11 and chlorina 14 which have reduced levels of Chl b, were used. Plants were grown in a greenhouse and thylakoid membranes were prepared from leaves of 30-days old seedlings as described previously (Terao et al. 1985a).

Pigments and quinones—Chl a, Chl b, pheophytin a, β-carotene, plastoquinone and vitamin K1 (phyloquinone) were extracted from thylakoids with ethanol and then with ethanol/diethyl ether (1:1) and analyzed with a Gilson HPLC system equipped with a TosO ODS-120T reverse-phase column. The mobile phase was 20% iso-propanol and 80% methanol and absorbance of eluent was monitored at 255 nm (Kato and Satoh 1988). Calibration curves were constructed with authentic samples. Chl a, Chl b and plastoquinone were extracted from spinach, purified with a large scale reverse-phase column and spectrophotometrically quantified (Mackinney 1941, Barr and Crane 1971). Pheophytin a was prepared by treating Chl a with 0.1 M HCl, purified by HPLC and determined at 669 nm with the absorption coefficient of 49 mM⁻¹ cm⁻¹ (in benzene). β-Carotene and vitamin K1 were purchased from Sigma. β-Carotene in hexane was estimated at 452 nm with the absorption coefficient of 139 mM⁻¹ cm⁻¹ (Zeichmeister and Polgar 1943). Vitamin K1 was quantified as described previously (Barr and Crane 1971).

Electron carriers—Mn was determined with a Hitachi 180-70 polarized Zeeman atomic absorption spectrophotometer. Thylakoid membranes were repeatedly washed with a hypotonic medium containing 0.2 M EDTA to remove contaminating Mn.

The herbicide-binding site was determined with 14C-atrazine according to the method described by Paterson and Arntzen (1982).

P-700 was assayed by measuring light-induced absorbance changes at 698 nm with a Shimadzu UV-3000 dual-wavelength spectrophotometer (Sonoike and Katoh 1990). The reaction media contained 50 mM Tricine-NaOH (pH 7.5), 2 mM MgCl₂, 10 mM NaCl, 5 mM ascorbate, 8 μM DCIP and 0.4 mM methyl viologen. Qa was quantified spectrophotometrically at 325 nm (Melis and Duysens 1979, Yamagishi and Katoh 1985). Photoreduction of Qa was carried out in the presence of 50 mM Tricine-NaOH (pH 7.5), 2 mM MgCl₂, 10 mM NaCl, 0.2 mM ferricyanide and 10 μM DCMU. Thylakoid membranes were treated with 0.04% Triton X-100 prior to measurement of absorption changes.

Oxygen evolution—Numbers of oxygen evolved per flash were determined with a Clark-type oxygen electrode. The reaction mixture contained 50 mM HEPES-KOH (pH 7.0), 2 mM MgCl₂, 10 mM NaCl, 20 mM methylamine, 1 mM ferricyanide and 0.4 mM phenyl-p-benzoquinone. Chl concentration was 10 μg ml⁻¹.

A dye laser (Phase RDL-1100) with Red 6 (oxazine 720) in ethanol was the source of 690 nm flashes with a half time of 300 ns. Flashes were fired at 1 Hz and amounts of oxygen evolved with 300 flashes were determined.

Fluorescence induction—Time courses of changes in the Chl a fluorescence yield were determined with a laboratory-constructed apparatus connected to a transient recorder. Thylakoids (10 μg Chl ml⁻¹) were suspended in 50 mM HEPES-KOH (pH 7.5), 10 mM NaCl, 5 mM MgCl₂ and 0.4 M sucrose and irradiated with blue or green light that was obtained by passing light from a halogen lamp through a Corning 4-96 filter alone or in combination with a Toshiba VO-36 cut-off filter. At the same photon flux density, blue light excites more Chl a and carotenoids than green light. The fluorescence emission at 688 nm was isolated with a Toshiba VR-64 cut-off filter, a 688 nm interference filter and a Bausch & Lomb monochrometer and measured with a Hamamatsu Electronics photomultiplier. Fluorescence induction in vivo was monitored with the same apparatus. A leaf segment was held diagonally to blue excitation light beam and emission at right angle to the excitation beam was detected.

Results

Contents of photosynthetic pigments and quinones in the thylakoid membranes from the wild-type and three mutant strains of rice were determined by HPLC. Elution profiles are shown in Fig. 1. Trace A indicates the presence of four pigments and two quinones in the normal thylakoids. The mutant membranes were strongly deficient in Chl b. No Chl b was detected in a Type I mutant, chlorina 2 (trace B), and the two Type II mutants, chlorina 11 (trace C) and chlorina 14 (trace D), showed smaller peaks of Chl b than did Norin No. 8.

![Fig. 1](https://example.com/fig1.png)
Table 1: Contents of photosynthetic pigments and quinones in the wild-type and three Chl b-deficient mutants of rice

<table>
<thead>
<tr>
<th>Strains</th>
<th>Norin No. 8</th>
<th>Chlorina 2</th>
<th>Chlorina 11</th>
<th>Chlorina 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl $a/b$ ratios</td>
<td>3.1± 0.2</td>
<td>—</td>
<td>9.4± 1.6</td>
<td>12.8± 3.3</td>
</tr>
<tr>
<td>Pheophytin $a$</td>
<td>8.7± 1.7</td>
<td>21.5± 2.0</td>
<td>18.4± 1.6</td>
<td>20.5± 2.0</td>
</tr>
<tr>
<td>(mmol (mol Chl)$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-Carotene</td>
<td>84 ±29</td>
<td>157 ±53</td>
<td>135 ±20</td>
<td>158 ±14</td>
</tr>
<tr>
<td>(mmol (mol Chl)$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plastoquinone</td>
<td>42 ± 2</td>
<td>82 ± 7</td>
<td>67 ± 3</td>
<td>82 ± 5</td>
</tr>
<tr>
<td>(mmol (mol Chl)$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin K$_{1}$</td>
<td>5.1± 0.4</td>
<td>8.7± 0.6</td>
<td>6.0± 0.5</td>
<td>8.1± 0.7</td>
</tr>
<tr>
<td>(mmol (mol Chl)$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe $a$/Vit K$_{1}$</td>
<td>1.71</td>
<td>3.47</td>
<td>3.08</td>
<td>2.51</td>
</tr>
</tbody>
</table>

Figures are means (±S.D.) of seven experiments.

Relative abundances of the pigments and quinones estimated are summarized in Table 1. A small peak which appeared after the $\beta$-carotene peak was included in the carotene fraction because of its absorption spectrum identical to that of $\beta$-caroten. Chl $a/b$ ratios of the wild-type, chlorina 11 and chlorina 14 were 3.1, 9.4 and 12.8, respectively, in agreement with the previous study, in which relative abundances of Chl $a$ and Chl $b$ were determined by fluorescence measurement at 77K (Terao et al. 1985a). The three mutants contained, on the basis of Chl, larger amounts of pheophytin $a$, $\beta$-carotene, plastoquinone and vitamin K$_{1}$ than Norin No. 8. This can be ascribed to low contents of Chl in the mutants because the relative abundance of each component increased with increasing deficiency of light-harvesting Chl $a/b$ proteins. All the components did not increase in parallel, however. Note that the pheophytin $a$ content was more than doubled in the mutants, whereas increases in abundance of vitamin K$_{1}$ were at most 70%. Thus, the mutants have larger ratios of pheophytin $a$ to vitamin K$_{1}$ than the wild-type rice. The PSI reaction center complex carries two molecules of vitamin K$_{1}$ (Omata et al. 1984). The elevated ratios of pheophytin $a$ to vitamin K$_{1}$ suggest, therefore, enrichment of PSII relative to PSI. When compared on the basis of vitamin K$_{1}$, the three mutants were also more abundant in plastoquinone, which serves as electron acceptor of PSII, and $\beta$-carotene, which is associated with the reaction center complexes of the two photosystems (Bassi et al. 1993) than Norin No. 8.

The vitamin K$_{1}$ content of 5.1 mmol per mol Chl suggests that there are about 2.5 mmol PSII per mol Chl in normal thylakoids. This value is larger than the PSI contents of about 1.6 mmol per mol Chl reported for other plants (Whitmarsh and Ort 1984, Chow and Hope 1987, Graan and Ort 1984). However, quantitation of P-700 also indicated a relatively high PSI content; there were 2.3 mmol P-700 per mol Chl in the thylakoids of Norin No. 8 (Table 2). Measurements of vitamin K$_{1}$ and P-700 showed that, on the basis of Chl, the chlorina mutants were more abundant in PSI than the wild type rice. As expected, the ratios of vitamin K$_{1}$ to P-700 were approximately two in all the mutant strains.

Table 2 also shows PSII contents determined by different methods. Q$_{A}$, the primary quinone acceptor of PSII, was estimated by measuring magnitudes of light-in-
duced absorbance changes at 325 nm (Melis and Duysens 1979, Yamagishi and Katoh 1985). Contents of QA in the three mutants were more than two times larger than that in the wild type rice on the basis of Chi. PSII was also quantified by measuring the herbicide-binding site with radioactive atrazine (Tischer and Strotmann 1977, Paterson and Arntzen 1982) and abundance of Mn, which is present at the ratio of 4 Mn/PSII center (Yocum et al. 1981). The PSII contents estimated by the two methods agreed with each other and also with those determined by photoreduction of QA, but were smaller than those estimated by assay of pheophytin a in all the four genotypes. Larger pheophytin a contents suggest pheophytinization of small amounts of Chl a during extraction and assay procedures.

PSII is heterogeneous in terms of electron transport and 10 to 40% of PSII centers have been reported to be unable to reduce the plastoquinone pool (Lavergne 1982, Melis 1985, Graan and Ort 1986, Govindjee 1990). We determined the active populations of PSII by measuring amounts of oxygen evolved per single-turnover flash. The flash was saturating even with chlorina 2 that totally lacks LHC-II and gave a constant yield of oxygen between 1 and 5 Hz. Abundances of PSII estimated from the amount of oxygen evolved per flash were significantly smaller than those obtained by quantitation of the functional components (Table 2), indicating the occurrence of inactive PSII centers in all the four genotypes. Non-functional PSII centers were particularly abundant in the mutants. The inactive population was 4 to 20% of the PSII centers in the normal thylakoids, whereas non-functional PSII amounted to about half the total PSII in the membranes isolated from the three chlorina mutants.

Relative abundance of the inactive PSII centers has been estimated by measuring fluorescence induction kinetics in dark-adapted thylakoids (Melis 1985, Cao and Govindjee 1990). This approach has yielded smaller estimates of the inactive PSII populations corresponding to 20–25% of the total PSII for Chl b-deficient mutants of other plants (Melis 1985, Ghiraridi and Melis 1988, Greene et al. 1988) than did measurement of oxygen evolution for the rice mutants. We analyzed, therefore, the fluorescence induction in the four rice genotypes (Fig. 2). On excitation, the fluorescence yield increased from the initial constant level (F0) to the intermediate plateau level (Fp), then followed by a larger increase to Fm, the maximum level. The rise from F0 to Fm was much slower in the mutant thylakoids than in the normal membranes, reflecting diminished antenna sizes of the mutant PSII. Fm level was also strongly reduced by the deficiency of Chl b.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Norin No. 8</th>
<th>Chlorina 2 (%)</th>
<th>Chlorina 11</th>
<th>Chlorina 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen evolution</td>
<td>4–20</td>
<td>54</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>Fluorescence induction</td>
<td>15</td>
<td>17</td>
<td>17</td>
<td>24</td>
</tr>
<tr>
<td>Fluorescence induction (leaf)</td>
<td>15</td>
<td>18</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>
Stoichiometry of PSI and PSII

Fig. 3 Fluorescence induction kinetics in a leaf blade of chlorina 2. The leaf sample was kept in the dark for 30 min prior to measurement. Blue excitation light of 400 μmol photons m⁻² s⁻¹ was turned on at time 0.

Fluorescence induction kinetics in leaves. Excitation light of above 200 μmol photons m⁻² s⁻¹ was used to ensure the full reduction of the plastoquinone pool. A typical fluorescence transients in a leaf blade of chlorina 2 is shown in Fig. 3. The F_r/F_v ratio determined in vivo was not much different from that obtained with isolated thylakoids. The percent abundances of the inactive PSII determined in vivo are 20% or less for other mutant strains (Table 3).

The difference could be explained if one assumes that the water-oxidation reaction was inactivated in a part of PSII centers during preparation of the mutant (but not normal) thylakoids. PSII centers that lost the water-oxidizing capacity do not contribute to the fluorescence yield rise because they are unable to accumulate reduced Q_A due to lack of electron supply to Q_A as well as rapid reoxidation of reduced Q_A. If 30–40% of PSII were damaged at the water oxidation site in the mutant membranes, therefore, the inactive PSII populations estimated by fluorescence measurement are still 10–20% of the total PSII, even though about 50% of the PSII centers are non-functional in electron transport. Experiments were carried out to examine whether the mutant (but not normal) thylakoids have a significant population of PSII centers with the water oxidation inactivated. Addition of Mn²⁺ that serves as an efficient electron donor to PSII had no or only a slight effect on the fluorescence yield changes in the mutant thylakoids (Fig. 4). Other PSII donors such as phenylenediamine were without effect and DCMU that blocks electron transfer from Q_A to Q_B failed to increase the fluorescence yield appreciably (not shown). There occurred some increase in the F_m level on the combined addition of Mn²⁺ and DCMU to the chlorina 2 thylakoids (Fig. 4). The magnitude of the yield increase was, however, not so large as would be expected if Q_A photoreduction were restored in 30–40% of PSII centers. The effect of Mn²⁺ plus DCMU was less significant in other chlorina strains (not shown). Thus, the large inactive PSII populations found in the mutant thylakoids cannot be ascribed to inactivation of the water oxidation during preparation of the membranes.

Discussions

The present study reports abundances of PSI and PSII in the wild-type and the three chlorina mutant strains of rice. The PSI contents determined by measuring P-700 and vitamin K₁ agree with each other and show that the mutants are 20 to 80% more abundant in PSI than the wild type rice on the basis of Chl. The differences are mainly ascribed to the reduced contents of Chl in the mutants. The result also shows that vitamin K₁ serves as a measure of PSI.

It is generally accepted that the herbicide-binding site is a good measure of PSII (Graan and Ort 1986, Chow et al. 1990). Determination of Mn gave the PSII contents that are comparable to those estimated by the specific binding of the radioactive herbicide, provided that extraneous Mn had been completely removed by repeated washes with a high concentration of EDTA. It has been disputed that quantitation of PSII by Q_A photoreduction at 325 nm has limitations that arise from heterogeneity of light-induced absorbance change and large flattening effects at this wavelength (Whitmarsh and Ort 1984, Jursinic and Dennenberg 1988). However, the PSII contents determined by Q_A photoreduction agreed with those estimated by the above two methods. Thus, the results show that all the PSII reaction center complexes present in the wild type and the three Chl b-deficient mutant strains carry one Q_A, one atrazine bind-
Table 4  Ratios of photosystem II to photosystem I

<table>
<thead>
<tr>
<th>Plant</th>
<th>(Q_{A}) P-700</th>
<th>(Q_{B}) site P-700</th>
<th>Mn \times \frac{1}{4} P-700</th>
<th>Phe \times \frac{1}{2} P-700</th>
<th>(O_{2}) yield P-700</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norin No. 8</td>
<td>1.30</td>
<td>1.09</td>
<td>1.17</td>
<td>1.92</td>
<td>1.04</td>
</tr>
<tr>
<td>Chlorina 2</td>
<td>1.86</td>
<td>1.79</td>
<td>1.81</td>
<td>2.55</td>
<td>1.00</td>
</tr>
<tr>
<td>Chlorina 11</td>
<td>2.48</td>
<td>2.01</td>
<td>3.27</td>
<td>3.30</td>
<td>1.32</td>
</tr>
<tr>
<td>Chlorina 14</td>
<td>2.23</td>
<td>2.27</td>
<td>3.14</td>
<td>3.32</td>
<td>1.19</td>
</tr>
</tbody>
</table>

ing site and four Mn.

For convenience of the following discussion, the ratios of PSII determined by the different methods to PSI (P-700) are shown in Table 4. PSII quantified by measurement of \(Q_{A}\). Mn or the herbicide-binding site is present more abundantly than PSI in all the four genotypes. The PSI to PSII ratio is 1.1–1.3 in the wild-type plant, 1.8 in chlorina 2 and above 2 in chlorina 11 and chlorina 14. Chl \(b\)-deficient mutants of other plants also have higher PSII to PSI ratios than the corresponding wild-type plants (Melis and Thielen 1980, Abadia et al. 1985, Ghirardi et al. 1986, Ghirardi and Melis 1988, Greene et al. 1988). An elevated PSII to PSI ratio is considered to be a response of plants to compensate the diminished antenna size of the mutant PSII.

As stated in Introduction, an elevated PSII to PSI ratio creates an imbalance in electron transport between the two photosystems. In strong light, larger numbers of electrons are delivered into the plastoquinone pool from water via PSII than are removed from the pool via PSI, resulting in 'overreduction' of the pool plastoquinone. This would reduce the light-utilization efficiency of photosynthesis by increasing a population of closed PSII centers with \(Q_{A}\) and consequently render PSII strongly susceptible to the harmful effect of light. In this respect, of special interest is the observation that the mutants have larger proportions of the inactive PSII than the wild type plant. Measurements of oxygen evolution per flash showed that about half the total PSII centers are inactive in electron transport in the mutant thylakoids. Thus, when only the active populations of PSII centers are taken into account, the stoichiometries of the two photosystems are one to one in the mutant membranes (Table 4). This stoichiometry is not an apparent one that is derived from accumulation of reduced plastoquinone during repetitive flash illumination because the quinone acceptor employed receives electrons directly from PSII (Graan and Ort 1986, Sato et al. 1992). Thus, the results obtained here point to an interesting possibility that plants have an ability to alter, responding to the deficiency of Chl \(b\), the organization of PSII so as to balance operation of the two photosystems in terms of not only light absorption but also electron transport.

Analysis of the fluorescence induction suggested, however, smaller proportions of the inactive PSII in the mutant thylakoids than did measurement of oxygen evolution per flash. The difference cannot be ascribed to inactivation of the water-oxidizing reaction. No evidence was obtained indicating the occurrence of a sizable PSII population that is inactive in the water oxidation in the mutant thylakoids. Dissociation of Mn or the extrinsic 33 kDa protein is a main cause of inactivation of the water oxidation (Ghanotakis and Yocum 1985). However, all the PSII centers present in the mutant thylakoids carry four Mn per center (Table 2) and highly active oxygen-evolving PSII particles isolated from the thylakoids of a Type I mutant (chlorina 9) were shown to retain a full complement of the extrinsic 33 kDa protein (Shen et al. 1988). These data support the notion that all the PSII centers present in isolated mutant thylakoids are competent in the water oxidation reaction.

There are two uncertainties that are associated with the fluorescence analysis. First, the estimation of the inactive PSII by this approach premises an identical emission yield between the active and inactive populations of PSII. There is evidence suggesting that some difference exists in the emission yield between PSII\(a\) and PSII\(b\) in the normal thylakoids which may correspond to the active and inactive PSII centers, respectively (Lee et al. 1990). Comparison of the four rice genotypes showed that the fluorescence yield is strongly reduced by the deficiency of Chl \(b\) or LHC-II. How the emission yields of the active and inactive PSII are influenced by the deficiency or lack of LHC-II is not known. Thus, it remains to be investigated whether the F\(a\)/F\(p\) ratio is linearly proportional to the ratio of the inactive PSII over the total PSII in the mutant genotypes. Second, the method assumes that the F\(a\) to F\(p\) rise originates from PSII\(a\) that is unable to reduce the plastoquinone pool (Melis 1985). Hsu and Lee (1991) have resolved the fluorescence induction of DCMU-poisoned thylakoids into three kinetic phases, a major rapid sigmoidal phase and two minor slower exponential phases. Unexpectedly, the rate constants of the two slower phases, of which a larger and faster one most likely corresponds to PSII\(b\), are independent of the intensity of excitation light and increased significantly by addition of hydroxylamine. Thus, more experiments are needed to understand the minor PSII population(s).
The measurement of oxygen evolution per flash yields more reliable estimates. The single turnover flashes used are saturating even for chlorina 2 that lacks LHC-II. The flash repetition rate of 1 Hz ensures the full restoration of the functional state of PSI. As far as normal thylakoids are concerned, the proportion of the active PSIII determined from the flash yield of oxygen agrees with those reported in the literature (Graan and Ort 1986, Chylla et al. 1987, Chylla and Whitmarsh 1989). The accompanying paper will show that the inactive PSIII are unable to contribute to steady state of oxygen evolution in strong light, either, but have an important function in the light-harvesting capacity of PSI (Terao and Katoh 1996). We suggest, therefore, that the abundance and organization of the photosystems are altered in a way to balance light absorption and electron transport between the two photosystems in the chlorina mutants that have diminished PSII antenna.

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