An Evidence Indicating That a Weak Orange Light Absorbed by Phycobilisomes Causes Inactivation of PSII in Cells of the Red Alga *Porphyridium cruentum* Grown under a Weak Red Light Preferentially Exciting Chl *a*

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Changes in the PSII fluorescence upon shift of light quality were studied with the red alga *Porphyridium cruentum* IAM R-1 and supplementarily with *P. cruentum* ATCC 50161, the cyanophytes *Synechocystis* spp. PCC6714 and PCC6803 and *Synechococcus* sp. NIBB1071. When *Porphyridium cruentum* grown under a weak red light (PSI light) preferentially absorbed by Chl *a* was illuminated with a weak orange light (PSII light) mainly absorbed by phycobilisomes (PBS), a change of PSII fluorescence at room temperature was induced. The ratio of Fvm (Fm — Fo) to Fm was reduced rapidly accompanying the increase in Fo (T(1/2), ca. 3 min). The effects of DCMU and 2,5-dibromo-3-methyl-6-isopropyl-β-benzoquinone indicated that the fluorescence change is induced when plastoquinone pool is highly reduced. The fluorescence change after a short PSII light illumination was reversible; it rapidly recovered in the dark (T(1/2), ca. 3 min). The reversibility was gradually reduced and disappeared after 40 h under PSII light accompanying decrease in PSII activity per PBS down to almost 50%. Since the pattern of the fluorescence change resembles that observable when PSII is photoactivated, PSII light probably induces the photoinactivation of PSII, possibly reversibly at first and irreversibly after prolonged illumination. Such a rapid fluorescence change was insignificant in *Synechocystis* sp. either PCC6714 or PCC6803. Only a slow and small decrease in Fvm/Fm level appeared after prolonged PSII light illumination (the reduction of PSII activity per PBS was around 20%). In *Porphyridium*, shift from PSII light to PSI light caused a rapid and chloramphenicol-sensitive Fvm/Fm elevation during the first 10 h while the increase in PSII activity per PBS was only 10% of that before the light shift. Then, a gradual elevation followed up to the level at the steady state under PSI light. A similar rapid increase in Fvm/Fm was observed with *Synechocystis* PCC6714, in which the synthesis of PSI is not regulated, suggesting that a rapid increase in Fvm/Fm does not reflect the acceleration of the synthesis of PSII. Results were interpreted as that (1) PSII light causes photoinactivation of PSII. Such a photoinactivation is marked in *Porphyridium* cells grown under PSI light. (2) In *Porphyridium*, changes in the abundance of PSII upon shift of light quality are largely attributed to the photoinactivation of this type.

Key words: Acclimation to light quality — Photoinactivation of PSII — Photosystem stoichiometry — *Porphyridium cruentum* — PSII fluorescence — Red algae.
(Cunningham et al. 1990) and Porphyry and the marine Synechococcus (Abe et al. 1993) whereas it is unity in Synechocystis spp. PCC 6714 (Ohki et al. 1987) and probably PCC6803, we assumed that in the former case, the amount of PSII is flexible and changes in response to the light quality (cf. Abe et al. 1993). However, the regulatory mechanism for changes in the amount of PSII has remained unknown. Does the regulation occur in the synthesis of PSII complex similarly to the regulation of the amount of PSI? Or, is the degradation of PSII regulated? Or, both? How is the synthesis and/or the degradation controlled? There remain many questions.

To know the mechanism, changes in the fluorescence from PSII upon shift of light quality were studied with the red alga P. cruentum IAM R-1 strain. The results reported herein indicated that (1) a rapid photoinactivation of PSII probably followed by degradation occurs in P. cruentum after the light shift from PSI light to PSII light, (2) such an inactivation is insignificant in Synechocystis sp. PCC6714, but (3) a slow and small decrease in the active PSI occurs after prolonged illumination with PSII light. The feature suggested that in Porphyridium cells, decrease in PSII after the shift to PSII light is attributed to the photoinactivation of PSII followed by degradation. Further, such a photoinactivation of PSII under PSII light may also contribute to changes in photosystem stoichiometry in Synechocystis sp. PCC6714 even though the contribution is minor.

**Material and Methods**

**Algal strains and cultures—**Strains of the red alga P. cruentum, IAM R-1 and ATCC50161—were grown in modified ASP2 medium (Provasoli et al. 1957); NaNO₃ and K₂HPO₄ were added at 100 mg per liter and 25 mg per liter, respectively. Na₂SiO₃ was omitted. Marine Synechococcus sp. NIBB10171 was grown in the modified ASP7 medium (Provasoli and Provasoli 1958); glycerophosphate was replaced with Na₂HPO₄ at 24 mg per liter and NaNO₃ concentration was increased up to 0.5 g per liter. Na₂SiO₃ was omitted. The cyanophytes Synechocystis spp. PCC6714 and PCC6803 were grown in MDM medium (cf. Watanabe 1963). Culture temperature was 25°C, except for P. cruentum ATCC50161 (20°C). The air containing ca. 1% CO₂ was continuously supplied. For the illumination exciting mainly Chl a, so that PSI, a weak red light longer than 660 nm was used (PSI light); the light from tungsten lamp was filtered through a combination of red (Mitsubishi Rayon 102) and blue (Shumitomo Chemicals 703) plastic filters (cf. Fujita et al. 1985); the light intensity, 52 μmol m~² s~¹ for the red algae and 20 μmol m~² s~¹ for two strains of Synechocystis. Marine Synechococcus was grown at 52 μmol m~² s~¹. For the illumination mainly exciting PBS, so that PSII, a weak orange light obtained by a combination of the fluorescent light (daylight type) and orange plastic filter (Asshi Chemicals 305, cf. Fujita et al. 1985) was used (PSII light); the intensity for cultures of Porphyridium, 18 μmol m~² s~¹, and for Synechocystis and the marine Synechococcus, 16 μmol m~² s~¹. The intensity was set so as to give the same growth rate under two light conditions, PSI light and PSII light, in cultures of respective organisms (doubling time, 35 ± 5 h for Porphyridium and 20 ± 3 h for Synechocystis. Growth of the marine Synechococcus in the present experiment was far slower than that in our previous study. Improvement has not been successful). Cell growth was monitored by the absorbance at 750 nm determined by a Hitachi 100-50 spectrophotometer. Cells at the early to middle exponential growth phase were used for the experiments. Absorption spectra of intact algal cells were measured by a Hitachi U3000 spectrophotometer with head on photomultiplier.

**Determination of the in vivo fluorescence emitted from PSII—**Determination of the fluorescence from PSII was performed at room temperature in two ways. (1) The maximum fluorescence (Fm) with DCMU (10⁻⁴ M) and the initial fluorescence rise (Fo) without DCMU were measured by a hand-made fluorometer; the fluorescence from cells (wavelengths longer than 670 nm) upon excitation of PBS (560 nm for Porphyridium and marine Synechococcus; 600 nm for Synechocystis) with monochromatic light prepared by a Ritu Optics monochromator MC-20L with halogen lamp light was recorded on a Kawasaki TMR-10 transient memory. The excitation intensity was 60 μmol m⁻² s⁻¹ for 560 nm light and 65 μmol m⁻² s⁻¹ for 600 nm light. Fo signals were stored at 10 μs per word, and for Fm, 5-10 ms per word; total words, 2000. (2) For monitoring the change of photosysost stoichiometry, the maximum fluorescence at 685 nm was determined in the presence of DCMU (10⁻⁴ M) and under excitation of Chl a (455 nm excitation) by a Hitachi MPF-4 spectrofluorometer. The fluorescence intensity on the basis of Chl a is roughly proportional to PSII/PSI ratio (Fujita et al. 1987b). Our preliminary examination for the relationship between the fluorescence index and the photosystox stoichiometry indicated that the index shown by P. cruentum IAM R-1 grown under PSI light was 3 times greater than that shown by the cells grown under PSII light (cf. Fig. 2D, 4). Since the ratio between PSII/PSI values of cells of the two types was around 6 (see below), the fluorescence index seems to be proportional to the photosystem stoichiometry by a factor of 1/2. Each determination was made, in principle, after the dark incubation for 30 min so as to remove the fluorescence change due to the state transition. However, the fluorescence levels in cells grown under either PSI light or PSII light were not altered by the dark incubation when determination was made after the illumination with PSI light. This indicates that the fluorescence level determined by the state transition under PSI light is the same as that after the dark incubation. Thus, on chasing the fluorescence change under PSI light, determination was made without dark incubation. The cell concentration for fluorescence determination was equivalent to 0.03 to 0.05 Abs.⁰

**Determination of the activity of photosystem II and photosynthesis—**The activity of PSI was determined by DMBQ- or DCBQ-Ferri Hill reaction. Cells were washed once with and suspended in the fresh culture medium at the concentration equivalent to 0.6-0.7 absorbance at the absorption maximum of maximum. The activity of Porphyridium cells was determined by the O₂ evolution in the presence of 1.2 mM DMBQ and 1.2 mM Ferri at 25°C. Illumination was made with a green light absorbed mainly by PBS (filtered through a Corning 4-72 and a Toshiba OS3; light source, 300 W halogen lamp; 16 μmol m⁻² s⁻¹). For the activity of Synechocystis cells, 0.5 mM DCBQ and 0.5 mM Ferri were added; light and temperature conditions were the same. The reaction was monitored with a Clark-type O₂ electrode (Hansatech). Since the rate limitation at the electron transfer from QA, primary quinone electron acceptor of PSII, to the added electron acceptor may cause inactivation of PSII (see below), the reactions were measured under light-limiting conditions. Measurements were done after the dark incubation for 30 min. The Hill
activities determined under these conditions were thought to correspond to the activity of PSII under the assumption that the energy transfer from PBS to PSII is the same in cells grown under either PSII light or PSI light.

Photosynthetic activities of Porphyridium cells were determined with O₂ evolution under PSI light (52 μmol m⁻² s⁻¹) at 25°C.

Results and Discussion

Short term experiments for the fluorescence change induced by the shift of light regime—A noteworthy fluorescence change was observed with cells of Porphyridium cruentum IAM R-1 grown under PSI light when the light regime was shifted to PSII light (Fig. 1A); levels of the maximum variable fluorescence (Fm − Fo, Fvm) divided by Fm, Fvm/Fm, were rapidly reduced upon shift of light regime. At the same time, Fo levels significantly increased. Decrease in Fvm/Fm levels was rapid in the first 10 min after the light shift (the half time, around 3 min; Fig. 1A, inset). The decrease then slowed down but continued until the level reached to that in cells grown under PSII light. The amplitude of the initial rapid decrease was 14 to 18% of the level before the light shift. Since the level in cells grown under PSII light (Fvm/Fm, 0.5–0.6) was around 75% of that in cells grown under PSI light (Fvm/Fm, 0.65–0.75), the rapid decrease was as great as 60 to 70% of the difference between levels in the two types of cells. Fvm/Fm levels somewhat varied among different cultures under the same conditions; variation of Fo appeared to be a main cause. The variation did not correlate with growth phase. Cells grown under PSII light did not show such a change when cells were transferred to experimental incubation under either PSI light or PSII light.

The same change was also observed with Porphyridium cruentum ATCC50161 and the marine Synechococcus sp. NIBB1071 whereas Synechocystis sp. either PCC 6714 or PCC 6803 did not show such a fluorescence change (Table 1). Although the decrease in Fvm/Fm in the marine Synechococcus sp. NIBB1071 was small, the decrease after the shift to PSII light was distinct. A low Fvm/Fm of the cells grown under PSI light suggests that a significant fraction of the cell population had been probably poor in photosynthetic activity. Since the shift from PSI light to PSII light for algal growth induces a decrease in the amount of PSII in P. cruentum ATCC 50161 (Cunningham et al. 1990) and the marine Synechococcus sp. NIBB1071 (Abe et al. 1993) but not Synechocystis spp. PCC 6714 and PCC6803 (cf. Fujita 1997), the fluorescence change appears to correlate with the decrease in the amount of PSII under PSII light.

As shown in Fig. 1B, the fluorescence change observed was reversible. Reduced Fvm/Fm under PSII light rapidly

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**Fig. 1** Changes in PSII fluorescence upon shift from PSI light to PSII light for cell growth (A) and dark recovery of the fluorescence change (B) observed with Porphyridium cruentum IAM R-1. In A, cells grown under PSI light were transferred to experimental incubation under PSII light (time zero). Values of Fvm/Fm were plotted against time after the light shift. Measurements at time zero were done after the dark incubation for 30 min; those after the shift to PSII light, without dark incubation. Inset indicates the time course of the fluorescence change during the first 15 min; the two time courses were obtained from experiments different from each other. In B, PSI light-grown cells were illuminated with PSII light for 50 min and transferred to the dark conditions. The upper open circle at time zero in B indicates the Fvm/Fm level before PSII light illumination. Open circles, Fvm/Fm; closed circles, Fo (relative to that at time zero). Experimental details, see the text.
Photoinactivation of PSII in *Porphyridium cruentum*

Table 1 Decrease in Fvm/Fm induced by a weak PSII light observed with various algal strains

<table>
<thead>
<tr>
<th>Algal strains</th>
<th>Fvm/Fm Before PSII light illumination</th>
<th>Fvm/Fm After PSII light illumination</th>
<th>ΔFvm/Fm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Porphyridium cruentum</em> ATCC50161</td>
<td>0.72</td>
<td>0.61</td>
<td>0.15</td>
</tr>
<tr>
<td><em>Synechococcus</em> sp. NIBB 1071</td>
<td>0.36</td>
<td>0.33</td>
<td>0.08</td>
</tr>
<tr>
<td><em>Synechocystis</em> sp. PCC6714</td>
<td>0.55</td>
<td>0.55</td>
<td>0.00</td>
</tr>
<tr>
<td><em>Synechocystis</em> sp. PCC6803</td>
<td>0.52</td>
<td>0.52</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Cells exponentially growing under PSI light (Before) were transferred to conditions under PSII light and incubated for 60 min (After). Values of ΔFvm/Fm are expressed as the difference between Fvm/Fm values before and after PSII light illumination relative to Fvm/Fm before the illumination. Experimental details, see the text.

Increased, accompanying decrease in Fo, in the dark to the levels before the illumination with PSII light. CAP did not suppress the recovery (data not shown) indicating that protein synthesis depending on chloroplast genome is not involved in the process. The rapid recovery appeared to be little less than the initial level, and the level depended on the illumination period with PSII light; lower level after longer illumination. Red light also appeared to cause a similar rapid recovery (data not shown).

According to our previous study with the cyanophyte *Synechocystis* sp. PCC6714 (Fujita et al. 1987a, Murakami and Fujita 1991), the shift from PSI light to PSII light causes over-reduction of electron transport components before Cyt b6f complex including PSII in cells of low PSI/PSII ratio grown under PSI light. The same may occur in *Porphyridium* cells, and the fluorescence change may correlate with such an electron transport state. DCMU reduces number of active PSII. Thus, DCMU is expectable to lower the reduced state of PQ pool even after the shift to PSII light. Table 2 shows the effect of reduction of active PSII by a partial DCMU-inhibition. DCMU accelerated fluorescence-rise so that determination of Fo size became somewhat less accurate. However, decrease in Fvm/Fm under PSII light was distinctly suppressed by DCMU suggesting that the fluorescence change is induced when PQ pool becomes highly reduced state. To confirm this

Table 2 Effects of DCMU and DBMIB on the fluorescence change induced by PSII light observed with *Porphyridium cruentum* IAM-R-1 grown under PSI light

<table>
<thead>
<tr>
<th>Addition</th>
<th>ΔFvm/Fm</th>
<th>Fo</th>
<th>Photosynthesis (relative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCMU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.131</td>
<td>1.20</td>
<td>1.00</td>
</tr>
<tr>
<td>1.5 x 10^{-8} M</td>
<td>0.054</td>
<td>1.02</td>
<td>0.55</td>
</tr>
<tr>
<td>3.0 x 10^{-8} M</td>
<td>0.017</td>
<td>1.00</td>
<td>0.35</td>
</tr>
<tr>
<td>DBMIB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>5.0 x 10^{-6} M</td>
<td>0.115</td>
<td>1.15</td>
<td>0.33</td>
</tr>
<tr>
<td>5.0 x 10^{-6} M, dark</td>
<td>0.011</td>
<td>1.00</td>
<td>—</td>
</tr>
</tbody>
</table>

Determination of Fvm/Fm at time zero was done after the dark incubation for 30 min, which reduced the fluorescence yield to the level under PSI light. In th experiments for DCMU effect, Fvm/Fm after the incubation under PSI light for 24 min was determined without dark incubation. In the experiments for DBMIB effect, cells were illuminated with PSI light with and without DBMIB, and Fvm/Fm levels were determined at appropriate time intervals for drawing the time course profile. The initial faster change (T1/2, ca. 1 min) was removed from the time course under assumption that the time course was composed of two monomolecular kinetics, and ΔFvm/Fm was determined from the slower change after PSI light illumination for 24 min. Expression of ΔFvm/Fm is the same as that in Table 1. Fo is expressed as the value relative to that at time zero. Other experimental details, see the text.
assumption, DBMIB effect was also examined. DBMIB blocks PQH₂ oxidation by Cyt b₅f. Thus, addition of DBMIB at low concentration must cause a high level of PQH₂ even under PSI light. Addition of DBMIB caused the state transition, so that decrease in the fluorescence level, even without light shift. However, the state transition, decrease in the fluorescence, due to DBMIB action was very rapid; T₁/₂ under PSI light was less than 1 min. Thus, the fluorescence change due to the state transition could be kinetically removed. Indeed, DBMIB induced a rapid decrease in Fₘ/Fₘ, together with increase in F₀, even under PSI light (Table 2). DBMIB itself quenches chlorophyll fluorescence. However, Fₘ/Fₘ decreased only in the light; no fluorescence change observed after the dark incubation. Results shown in Table 2 must be safe from such quenching effect. The effect of the two electron transport inhibitors indicates that (1) the state for over-reduction of PQ pool induces the reversible fluorescence change in Porphyridium cells grown under PSI light whereas (2) the reduced state of QA in closed PSII induced by DCMU at low concentration is not responsible to the fluorescence change.

Since the fluorescence change occurs upon over-reduction of PQ pool, the change may relate to changes in the state at the reducing side of PSII. Decrease in Fₘ/Fₘ resembles the fluorescence change during the photoinactivation of PSII under strong light (Krause 1994, Yokoyama et al. 1991). However, the simultaneous increase in F₀ and the reversibility of the fluorescence change, which are not expected to be observable under our aerobic experimental conditions (cf. Styring and Jegerschold 1994), also occurred. Although the pattern of the fluorescence change leads us to assume an occurrence of an abnormal state of PSII which results in the inactivation of the system, the event reflected by the fluorescence change may not be simply analogous to that occurring in the photoinactivation of PSII under strong light.

Inspite of a similarity of the fluorescence change to that for the photoinactivation of PSII under strong light, the inactivation of PSII could not be observed so far as examined with the DMBQ-Ferri Hill activity (data not shown). Possibly, the rapid dark recovery did not permit us to observe the inactivation. Since the level of the dark recovery tended to be lowered after longer illumination with PSI light, the reversibility of the fluorescence change and the activity of PSII were examined at much longer time length.

Long term experiments for fluorescence change induced by the shift of light regime—The change of Fₘ/Fₘ in P. cruentum IAM R-1 was pursued for a period over one generation after the shift from PSI light to PSII light. The level of Fₘ/Fₘ was measured with a portion of cell suspension immediately after sampling. Then remaining cell suspension was incubated in the dark for 30 min, and Fₘ/Fₘ was again determined. The amplitude of the reversibility was derived from the difference between the two

![Graph](https://academic.oup.com/pcp/article-abstract/40/9/924/1940182/190182)
measurements. The activity of PSII was also determined by the DMBQ-Ferri Hill reaction.

Increase in cell density, PBS and Chl a after the shift from PSI light to PSII light are shown in Fig. 2A-C. After a long lag time (around 20 h), cells increased exponentially (Fig. 2A). The same pattern was observed with PBS as indicated by A_{375} (Fig. 2B). Chl a (indicated by A_{680}) increased without lag time and with a rate little faster than the former (Fig. 2C). The level of Fvm/Fm was reduced rapidly (cf. Fig. 1A). The second plot for the open circle in Fig. 2D was the Fvm/Fm level after illumination with PSII light for 30 min. The level was further reduced gradually with time under PSII light (Fig. 2D, open circles). The reduced level was elevated by the dark incubation (Fig. 2D, closed circles) indicating that the reduction is reversible. However, the dark elevation became smaller with time and was insignificant after 40 h. The maximum fluorescence under Chl a excitation, an index for PSII/PSI ratio, showed a similar pattern to that of Fvm/Fm after the dark incubation (Fig. 2D, open triangles) indicating that a rapid stoichiometry change occurred during this period. The PSII activity per PBS, determined by DMBQ-Ferri Hill activity and A_{375}, was reduced to two thirds of the initial after 20 h under PSII light, and to almost half after 40 h; the latter level was little higher than that in cells steadily growing under PSI light (Table 3). Determination of PSII activities was made in the experiment different from that for Fvm/Fm. However, cell growth and PBS increase in the former were the same as those in the latter (data not shown). The increase in PBS during the first 20 h is insignificant (Fig. 2B) indicating that the reduction of PSII activity observed during this period is attributed to the inactivation of existing PSII. Such an evidence indicates that the shift of light quality from PSI light to PSII light induces photo-inactivation of PSII in Porphyridium cells even under a weak light. Inactivation of PSII under a weak light was reported by Ohad et al. (1994) and analysed by Keren et al. (1997) although the mechanism of photoinactivation found with Porphyridium is not necessarily the same as that proposed by them.

According to Cunningham et al. (1990), the abundance of active PSII in Porphyridium cells grown under PSI light is double of that in cells grown under PSI light. This must be true in our case also since PSII activity per one PBS in cells grown under PSI light was almost half of the activity in cells grown under PSI light (Table 3). Preliminary spectroscopic determination of the stoichiometry among PSI, PSII and Cyt b_{6}f in Porphyridium cruentum IAM R-1 showed the ratio, 3 : 1: 1 in cells grown under PSI light, and 1 : 2 : 1, grown under PSII light (unpublished data). Thus, the decrease in the active PSII to the level in cells grown under PSI light almost completed during the first 40 h after the shift to PSII light. The reduction of PSII activity per PBS after the shift to PSII light is probably attributed to mainly the photoinactivation; retardation of synthesis of PSII upon shift to PSII light, if any, may have a minor effect on the decrease in PSII per PBS. Under PSI light, the repair of inactivated PSII may not compensate the inactivation. The inactivated PSII may be degraded, and the abundance of PSII complex has to be reduced as observed in the preliminary spectroscopic determination of photosystem stoichiometry.

The same experiment was conducted with Synechocystis PCC6714. A rapid decrease in Fvm/Fm found with P. cruentum did not occur in Synechocystis (Fig. 3A, open circles). During the initial 10 h under PSII light, the level was almost constant. Further illumination with PSII light caused a gradual decrease down to the level at 80 to 90% of the initial. The PSII activity per PBS of the cells grown under PSI light was around 80% of that of cells grown under PSI light (Table 3). A slow decrease of Fvm/Fm found with Synechocystis PCC6714 is probably an appearance of inactivation of PSII during the prolonged illumination though the amplitude is minor. Results indicate that (1) a rapid decrease in Fvm/Fm is induced by the shift to PSII light specifically in Porphyridium cells while a slow decrease is commonly induced in both Porphyridium and Synechocystis cells. (2) Inactivation of PSII corresponding to the former is far greater than the inactivation reflected by the latter.

Table 3 Changes in PSII activities of cells of Porphyridium cruentum IAM R-1 and Synechocystis PCC6714 grown under PSI light after the light shift from PSI light to PSII light

<table>
<thead>
<tr>
<th>Time after shift to PSII light (h)</th>
<th>Porphyridium cruentum IAM R-1</th>
<th>Synechocystis sp. PCC6714</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>20</td>
<td>0.65</td>
<td>—</td>
</tr>
<tr>
<td>40</td>
<td>0.57</td>
<td>—</td>
</tr>
<tr>
<td>100&lt; a</td>
<td>—</td>
<td>0.80</td>
</tr>
<tr>
<td>200&lt; a</td>
<td>0.55</td>
<td>—</td>
</tr>
</tbody>
</table>

Cells grown under PSI light were transferred to conditions under PSII light. Hill activities per PBS are expressed as the activities based on A_{375} of cell suspensions for Porphyridium and A_{680} for Synechocystis. Activities of Porphyridium were determined as DMBQ-Ferri Hill activities, and those of Synechocystis, DCBQ-Ferri Hill activities. Values are expressed as those relative to that of cells grown under PSI light. Activities at time zero were 0.641 mmol O_{2} A_{680}^{-1} ml^{-1} h^{-1} for Porphyridium and 0.174 mmol O_{2} A_{680}^{-1} ml^{-1} h^{-1} for Synechocystis cells. Other experimental details, see the text.

* Longer than 5 generation times.
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Fig. 3 Changes in Fvm/Fm in Synechocystis PCC6714 cells after the shift of light quality. In A, cells grown under PSI light were transferred to conditions under PSII light at time zero. In B, the light quality was shifted in reverse direction. Open circles, Fvm/Fm; open triangles, PSII/PSI index (relative to the initial; the initial PSII/PSI ratio, 0.4 for A and 0.8 for B). Experimental details, see the text.

The reduction of PQ pool induced by the shift to PSII light causes reversibly an abnormal state at the reducing side of PSII, which leads PSII to inactivation with passing the time under PSII light. This assumption is supported by the evidence that in Synechocystis, neither a marked inactivation of PSII nor the reversible fluorescence change is observable (Fig. 3A vs. Table 3).

The experiments for a reverse light shift, from PSII light to PSI light, were done with Porphyridium IAM R-1 (Fig. 4) and Synechocystis PCC6714 also (Fig. 3B). In Porphyridium, Fvm/Fm increased rapidly during the initial 10 h, after then slowed down; the increase almost ceased after 70 h (Fig. 4, open circles). The level attained during the first 10 h was more than 50% of the difference between levels of PSI light-grown and PSII light-grown cells. The initial rapid increase of Fvm/Fm, probably slow one also, was inhibited by CAP, indicating that such an increase depends on protein synthesis (Fig. 4, closed circles). The levels of Fvm/Fm elevated after the shift to PSI

Table 4 Changes in PSII activity as indicated DMBQ-Ferri Hill activity in cells of Porphyridium cruentum IAM R-1 grown under PSII light after shift to conditions under PSI light.

<table>
<thead>
<tr>
<th>Time after shift to PSI light (h)</th>
<th>PSII activity per PBS (relative)</th>
<th>Fvm/Fm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
<td>1.05</td>
<td>1.07</td>
</tr>
<tr>
<td>10</td>
<td>1.11</td>
<td>1.12</td>
</tr>
</tbody>
</table>

Cells grown under PSII light were transferred to conditions under PSI light. Hill activities per PBS are expressed as the activities based on A_{570} of cell suspensions. Values of Hill activity per PBS and Fvm/Fm are expressed as those relative to the initials, respectively. Fvm/Fm after reaching steady state was around 1.2. The initial Hill activity was 0.357 μmol O₂ A_{570}⁻¹ ml⁻¹ h⁻¹. Experimental details, see the text.
light were reversibly reduced by the illumination with PSII light (30 min) down to the level at time zero, and the subsequent dark incubation for 30 min elevated the level up to almost the same as that before the illumination with PSII light (data not shown). The initial rapid increase of Fvm/Fm is dramatic. However, elevation of the DMBQ-Ferri Hill activity was only 10% of the initial (Table 4). The increase in the index for PSII/PSI ratio continued almost linearly up to 60 h, and then slowed down (Fig. 4, open triangles). These features indicate that the increase in active PSII indicated as the initial Fvm/Fm rise is small so that it cannot cause a significant change of photosystem stoichiometry.

A similar rapid increase in Fvm/Fm was also found with Synechocystis sp. PCC6714 upon shift to PSI light (Fig. 3B). Thus, a rapid increase of Fvm/Fm after the shift to PSI light is common in Porphyridium and Synechocystis cells. Since the synthesis of PSII in Synechocystis is not accelerated by PSI light (Aizawa and Fujita 1997), a rapid increase in Fvm/Fm does not necessarily indicate the accelerated synthesis of PSII. The character of a rapid Fvm/Fm increase may be different from the slow increase following the former although both depend on protein synthesis. The former may be attributed to a replacement of damaged D1 peptide, a peptide of PSII core complex, with newly synthesized one whereas the latter may be mainly a reflection of de novo synthesis of PSII complex. A common appearance of a rapid Fvm/Fm rise suggests that a small portion of PSII complexes remains irreversibly damaged, probably at D1 peptide, not only in Porphyridium but also in Synechocystis cells growing under PSI light.

General consideration—Based on the interpretation of the fluorescence change observed in the present experiment, we can draw a picture for dynamics of PSII upon shift of light quality. The shift from PSI light to PSII light probably causes a highly reduced state of PQ pool, which may induce an abnormal state at the reducing side of PSII. After prolonged illumination with PSII light, PSII at such a state may be inactivated, possibly due to damage of D1 peptide, and then may be degraded. Such a photoinactivation and degradation of PSII may occur commonly in oxygenic photosynthesis at least having PBS as the light harvester upon shift of light quality, from PSI light to PSII light, even though the light intensity is weak enough. However, the amplitude of this photoinactivation is quite different depending on organism. In Porphyridium, the amplitude of inactivation is very high (more than one thirds of PSII was inactivated during the first 20 h) whereas such an inactivation is almost insignificant in Synechocystis (even after prolonged illumination with PSII light, inactivated PSII was only 20%).

Therefore, the degradation triggered by the photoinactivation under PSII light may contribute to the smaller abundance of PSII in Porphyridium cells grown under PSII light. A prominent decrease in PSII levels without increase in PBS during the first 20 h after the shift to PSII light indicates that the degradation of this type may be a main cause for variation of the abundance of PSII in Porphyridium cells. Such a degradation of PSII is far smaller in Synechocystis so that we hypothesized previously the regulation of photosystem stoichiometry attributed to only the regulation of synthesis of PSI (cf. Fujita 1997). However, we now realize that changes in photosystem stoichiometry are resulted from not only the actively regulated synthesis of PSI but also the inactivation of PSII under PSI light and that the latter markedly affects the change of the photosystem stoichiometry in Porphyridium cells. However, this does not necessarily exclude a possibility for that the synthesis of PSII is actively regulated in Porphyridium. Such a possibility is now under investigation.

A question arises from the present study. Why is PSII in Porphyridium so sensitive to the shift of light quality? The following is one of possible answers. The shift to PSII light accelerates reduction of PQ by PSII. According to the study with Porphyra and the marine Synechococcus (Abe et al. 1993), the abundance of Cyt b$_{6}$f complex seems to be unaltered when the abundance of PSII is changed depending on the light quality so that the stoichiometry of PSII to Cyt b$_{6}$f is greater in cells grown under PSI light than that in cells grown under PSII light. As described above, our preliminary determination of PSII/Cyt b$_{6}$f ratio in P. cruentum IAM R-1 gave around 2 for cells grown under PSI light, and around 1, under PSII light (unpublished data). The latter stoichiometry is the same level as that in Synechocystis PCC6714; the stoichiometry of this cyanophyte is constant irrespective of the light quality (PSII/Cyt b$_{6}$f, around 1; Fujita and Murakami 1987). Thus, the electron input to PQ pool is twice greater than the output from the pool in Porphyridium cells grown under PSI light. Such a high stoichiometry suggests that the shift to PSI light causes an over-reduced state of PQ pool that leads to a far greater number of PSII to an abnormal state. Thus photoinactivation may occur at higher frequency in Porphyridium cells grown under PSI light. However, it is also possible that the difference in sensitivity is not simply attributed to the stoichiometry, PSII/Cyt b$_{6}$f, but a high sensitivity is attributed to a special architecture around PSII of Porphyridium, which appears only in cells grown under PSI light.
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