Changes in Photosystem Stoichiometry in Response to Environmental Conditions for Cell Growth Observed with the Cyanophyte *Synechocystis* PCC 6714

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Changes in photosystem stoichiometry in response to shifts of environments for cell growth other than light regime were studied with the cyanophyte *Synechocystis* PCC 6714 in relation to the change induced by light-quality shift. Following two environment-shifts were examined: the shift of molecular form of inorganic carbon source for photosynthesis from CO₂ to HCO₃⁻ (CO₂ stress) and the increase in salinity of the medium with NaCl (0.5 M) (Na⁺ stress). Both CO₂ and Na⁺ stresses induced the increase in PSI abundance resulting in a higher PSI/PSII stoichiometry. CO₂ stress was found to elevate simultaneously Cyt c oxidase activity (Vₙₐₓ). The feature was the same as that caused by light-quality shift from preferential excitation of PSI to PSII (light stress) though the enhancement by either stress was smaller than that by light stress. Under our experimental conditions, PSI/PSII stoichiometry appeared to increase at a fairly constant rate to the basal level even when the basal level had been differentially determined by the light-quality. Enhancing rates for PSI/PSII stoichiometry and for Cyt c oxidase activity were also similar to each other. Since the two stresses affect the thylakoid electron transport similarly to the shift of light-quality, we interpreted our results as follows: three environmental stresses, CO₂, Na⁺, and light stresses, cause changes in electron turnover capacity of PSI and Cyt c oxidase under a similar, probably a common, mechanism for monitoring redox state of thylakoid electron transport system.

**Key words:** Cyt c oxidase — Environmental stress — Photosystem stoichiometry — *Synechocystis* PCC 6714 — Thylakoids.

Photosystem stoichiometry in cyanophytes varies in response to light-quality for cell growth (Fujita et al. 1985, Manodori and Melis 1986). The variation maintains the photosynthetic efficiency under respective light-qualities (Murakami and Fujita 1988, Melis et al. 1989). Thus, together with the regulation of excitation-energy distribution to the two photosystems, "state transitions", the regulation of photosystem stoichiometry is balancing the two photosystem actions so as to maintain the efficiency of oxygenic photosynthesis (cf. Fujita et al. 1994).

Our previous study (Gu et al. 1994) has revealed that in the cyanophyte *Synechocystis* PCC 6714, light-quality shift induces changes in Cyt c oxidase activity in parallel to changes in PSI/PSII stoichiometry. Even when cells were grown autotrophically, the activity (Vₙₐₓ) varied by a factor of 2 upon light-quality shift from the light absorbed mainly by Chl a (PSII light) to that absorbed mainly by phycobilisome (PSI light). Since almost all of Cyt c oxidase locate in thylakoids in this organism (Wastyn et al. 1987, see also Schmetterer 1994), variation of Cyt c oxidase activity observed is ascribed to that of the enzyme in thylakoids. Thus, the light stress on photosynthesis probably induces regulation of the electron turnover capacity of the terminal component not only of photosynthetic electron transport system (ETS), PSI, but also of the respiratory ETS, Cyt c oxidase, in thylakoids simultaneously. Cyt c oxidase activity of cyanophytes has been known to increase when cells are grown in a high salinity medium with a high NaCl concentration (Na⁺ stress; Moltitor et al. 1986, Jeanjean et al. 1990, Moser et al. 1991). Simultaneous increase in the abundance of PSI was also observed (Schubert and Hagemann 1990, Jeanjean et al. 1993, Hibino et al. 1996). On the other hand, it has been known that photosystem stoichiometry in cyanophytes varies depending on the molecular form of inorganic carbon source for photosynthesis (Eley 1971, Manodori and Melis 1984). The ratio becomes higher when cells are grown with HCO₃⁻ as a main inorganic carbon source for photosynthesis (CO₂ stress). Since requirement of extra energy under either CO₂ (cf. Kaplan et al. 1994) or Na⁺ stress (Moltitor et al. 1986) may modify thylakoid ETS state like light-quality shift, we suspect that both Na⁺ and CO₂ stresses cause regulation of the electron turnover capacity of the two terminals of thylakoid ETS, PSI and Cyt c oxidase, under a mechanism similar to that for responding to the light stress.

To evaluate such a possibility, we determined effects of CO₂ and Na⁺ stresses on PSI abundance and on Cyt c oxidase activity under PSI light and PSII light. Results indicated that (1) either CO₂ or Na⁺ stress enhanced the abundance of PSI and the activity (Vₙₐₓ) of Cyt c oxidase in
parallel like the shift of light quality from PSI light to PSII light and that (2) the level elevated by either stress was determined by the light quality.

Materials and Methods

Organization and culture—Synechocystis PCC 6714 was used and grown in MDM medium (Watanabe 1960) after a slight modification as described in our previous paper (Gu et al. 1994). Culture temperature was 25°C. For autotrophic growth, cultures were illuminated with either an orange light absorbed by phycobilisome so that exciting mainly PSI (17 μE m⁻² s⁻¹, PSI light) or a red light absorbed mainly by Chl a and exciting mainly PSI (35 μE m⁻² s⁻¹, PSII light) (cf. Fujita et al. 1985) with a continuous supply of the air containing 5% CO₂. For CO₂ stress, cells were grown in the medium containing 5 mM NaHCO₃ with supply of the air washed with 0.1 M NaOH. The medium pH was maintained at 7.5 to 8.5 using a pH stat (pH electrode, Bradley-James Co., CA, U.S.A.; pH controller, ABLE PHC-22, Tokyo, Japan). The pH of the control cultures was maintained at 6.5 to 7.5. For Na⁺ stress, NaCl was added to the culture medium at 0.5 M. For heterotrophic growth, glucose was added at 30 mM, and cultures were shaken in a thermostatted reciprocal shaker. Cells at the late exponential growth phase were used for determination of thylakoid components. Cell density was determined by counting cell number on a hemocytometer under a light microscope.

Determination of thylakoid components—PSI, PSII and Cyt b₆/f were determined spectrophotometrically as described previously (Fujita and Murakami 1987). For determination of Cyt c oxidase activity (Vₘₐₓ), O₂ uptake by membranes isolated from cells grown in respective cultures was determined using a Clark-type O₂ electrode (YSI 4004) in the presence of mammalian Cyt c and an excess amount of ascorbate as described previously (Gu et al. 1994). The abundance and the activity were expressed on a per Chl a basis. Chl a contents in membranes and in cells were determined spectrophotometrically with acetone extracts using the absorption coefficient of Mackinney (1941).

Results and Discussion

Variation of abundance of PSI and Cyt c oxidase activity induced by CO₂ stress—PSI/PSII ratio is enhanced by CO₂ stress in cyanophytes (Eley 1971, Manodori and Melis 1984). We tried to confirm the effect of CO₂ stress with Synechocystis PCC 6714. However, when cells were grown with HCO₃⁻, the medium pH was markedly shifted to alkaline side during cell growth; the shift from 7.8 to more than 10 was observed at the end of exponential cell growth. Cultures with CO₂ supply also caused a pH shift to acidic side. However, the shift was less marked, less than 1. To minimize such a pH shift during the culture, we adopted a pH stat for experimental cultures.

First, we examined validity of our experimental conditions for determination of the effect of CO₂ stress. Results are shown in Table 1. Two cases under PSI light are presented; the case for cultures under the pH preferable to

| Table 1 Effect of CO₂ stress on photosystem stoichiometry and Cyt c oxidase activity in cells grown under PSI light and PSII light |
|-------------------|-----------------|-----------------|-----------------|
| Light regime and | Abundance of     |                   |                   |
| inorganic carbon  | PSI (10⁻¹⁷ mol | PSII (10⁻³ mol | Cyt b₆/f (mol Chl a⁻¹)|                      |
| source            | cell⁻¹)         | mol Chl a⁻¹)     |                   |                   |
| Under PSI light   |                 |                  |                   |                   |
| I. CO₂ (pH 7.5)   | 3.87 ± 0.16     | 6.21 ± 0.13 (1.60) | 6.32 ± 0.14 (1.63) | 3.04 ± 0.17       |
| HCO₃⁻ (pH 7.5)    | 3.51 ± 0.31     | 4.13 ± 0.10 (1.18) | 8.24 ± 0.14 (2.35) | 4.84 ± 0.091      |
| II. CO₂ (pH 6.7)  | 4.47 ± 0.15     | 6.04 ± 0.17 (1.35) | 7.56 ± 0.28 (1.69) | 3.46 ± 0.16        |
| HCO₃⁻ (pH 8.1)    | 3.07 ± 0.02     | 4.28 ± 0.06 (1.39) | 8.78 ± 0.23 (2.86) | 4.57 ± 0.09        |
| Under PSII light  |                 |                  |                   |                   |
| CO₂ (pH 6.7)      | 2.26 ± 0.11     | 2.62 ± 0.29 (1.16) | 8.42 ± 0.40 (3.73) | 8.09 ± 0.05        |
| HCO₃⁻ (pH 8.2)    | 1.76 ± 0.06     | 2.33 ± 0.19 (1.32) | 8.75 ± 0.36 (4.97) | 4.54 ± 0.05        |

Respective cultures were maintained at pH values in parentheses. Numbers in parentheses for PSI and Cyt b₆/f were values on a per PSII basis (mol mol⁻¹). Those for Cyt c oxidase activity, on a per Cyt b₆/f basis [mol O₂ uptake (mol Cyt b₆/f h)⁻¹]. Experimental details, see the text.
each form of inorganic carbon and the other for cultures
under the same pH. In either case, PSI/PSII stoichiometry
increased when HCO₃⁻ was a sole inorganic carbon source.
Thus, the effect was not resulted from the pH shift but
from the shift of inorganic carbon source.

In the same table, the effect under PSII light is also
presented. The elevation by the stress was smaller than that
induced by the light quality under either light regime. The
elevated level under PSI light was lower than that of the
control under PSII light. Although the increment due to
the stress was greater under PSI light than that under PSI
light, the enhancing rate, the ratio of the level under the
stress to that of control, was rather similar under the two
light regimes (see also Table 4).

Differently from PSI/PSII stoichiometry, the stoichi-
ometry of Cyt b₆f to PSII was independent of the stress.
The latter somewhat scattered but fluctuation did not corre-
late with the stress. Cyt b₆f/PSII stoichiometry probably
is not altered by the stress. If it is correct, the abundance of
PSI must vary in response to CO₂ stress as similar to the re-
sponse to the light-quality at least in this organism. As seen
in the same table, CO₂ stress increased Cyt c oxidase ac-
divity on a per Cyt b₆f basis, so that PSII basis, in parallel to
the increase in PSI abundance like the response to the light
quality. As similar to elevation of PSI/PSII stoichiometry,
the enhanced level under PSI light was always lower than the
control level under PSII light. However, the enhancing rates
under the two light regimes were similar to each other in
spite of a great difference of basal levels. Enhancing rates
were similar to those of PSI/PSII stoichiometry also
(cf. Table 4). The effect of CO₂ stress on Cyt c oxidase ac-
divity was very similar to that on the abundance of PSI.

Chl a content on a per cell basis in stressed cells grown
under PSII light was small (Table 1). Smaller content was
also observed in heterotrophic cultures under Na⁺ stress
(Table 3). Observation under a light microscope indicated
that the cell size in these cultures became distinctly smaller
than those of the control. A smaller Chl a content is proba-
ably ascribed to smaller cell size.

Variation of PSI abundance and Cyt c oxidase activity
induced by Na⁺ stress—As has been reported (cf. Wastyn
et al. 1987), Cyt c oxidase activity was enhanced by Na⁺
stress in our experimental culture also. At the same time,
we confirmed the increase in PSI/PSII ratio (Table 2).
Enhancement of PSI/PSII stoichiometry was also observed
under heterotrophic growth conditions (Table 3).

As similar to CO₂ stress, the stoichiometry between
PSII and Cyt b₆f appeared not to be altered by Na⁺ stress;
fluctuation did not correlate with the addition of the stress.
The stoichiometry is probably constant. If it is correct, the
increase in PSI/PSII ratio is ascribed to the increase in
the abundance of PSI. Again, the absolute increment under
PSII light was greater than that under PSI light, and the en-
hanced level under PSI light was far lower than that of the
control under PSII light. However, the rate of the level
elevated by the stress to the basal, the enhancing rate, was
fairly constant irrespective of the basal level like the en-
hancement due to CO₂ stress (cf. Table 4). Enhancing rate
of Cyt c oxidase activity was similar to that of PSI/PSII
stoichiometry (Table 2, 4).

Na⁺ stress under heterotrophic conditions with glucose
caused enhancement of PSI/PSII stoichiometry
(Table 3). Since the stoichiometry between PSI and Cyt b₆f,
was almost constant, the abundance of PSI increased upon
the stress. The enhancing rate was similar to that under
autotrophic conditions. However, elevation of photosys-
tem stoichiometry by Na⁺ stress disappeared when cultures
with glucose were illuminated with either PSI light or PSII

Table 2 Variation of PSI/PSII stoichiometry and Cyt c oxidase activity induced by Na⁺ stress in cells grown under PSI light and PSII light

<table>
<thead>
<tr>
<th>Growth conditions</th>
<th>Abundance of PSI</th>
<th>Cyt b₆f activity</th>
<th>Cyt c oxidase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PSII [10⁻³ mol (mol Chl a)⁻¹]</td>
<td>PSI [10⁻¹⁷ mol cell⁻¹]</td>
<td>activity [mol O₂ uptake (mol Chl a h)⁻¹]</td>
</tr>
<tr>
<td>Autotrophic, under PSI light</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.60±0.03</td>
<td>7.20±0.22 (1.57)</td>
<td>6.72±0.38 (1.46)</td>
</tr>
<tr>
<td>Plus 0.5 M NaCl</td>
<td>4.45±0.09</td>
<td>4.96±0.05 (1.11)</td>
<td>8.57±0.5 (1.93)</td>
</tr>
<tr>
<td>Autotrophic, under PSII light</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.25±0.10</td>
<td>2.46±0.03 (1.09)</td>
<td>8.82±0.21 (3.92)</td>
</tr>
<tr>
<td>Plus 0.5 M NaCl</td>
<td>1.68±0.07</td>
<td>2.06±0.20 (1.23)</td>
<td>10.00±0.28 (5.95)</td>
</tr>
</tbody>
</table>

Numbers in parentheses for PSI, Cyt b₆f and Cyt c oxidase activity are the same as those in Table 1. Experimental details, see the text.
light.

Mode of enhancement under three environmental stresses—Enhancing effects of the two stresses on the photosystem stoichiometry and on Cyt c oxidase activity are summarized in Table 4. Data in the table are not sufficiently comprehensive for determining quantitative relationship among effects of three stresses. However, they can indicate that the increment of PSI/PSII stoichiometry induced by either CO₂ or Na⁺ stress is not constant under different light regime but appears to be proportional to the basal level differently determined by the light quality. Light stress, changes in light quality, is probably synergistic to either of two other stresses suggesting a close relationship among effects of three stresses.

Further, photosystem stoichiometry varied being accompanied by variation of Cyt c oxidase activity in all cases. The latter, together with the former, was enhanced by CO₂ stress like by light stress even under photosynthetic condition. To the contrary, the former, upon Na⁺ stress, increased even under dark-heterotrophic condition. Mode

Table 4 CO₂ stress- and Na⁺ stress-induced enhancement of photosystem stoichiometry and Cyt c oxidase activity at different basal levels determined by light-quality

<table>
<thead>
<tr>
<th>Light regime</th>
<th>Photosystem stoichiometry</th>
<th>Cyt c oxidase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal level</td>
<td>Enhancing rate</td>
</tr>
<tr>
<td>CO₂ stress</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSI light I</td>
<td>1.0</td>
<td>1.4</td>
</tr>
<tr>
<td>PSI light II</td>
<td>1.0</td>
<td>1.7</td>
</tr>
<tr>
<td>PSII light</td>
<td>2.4</td>
<td>1.3</td>
</tr>
<tr>
<td>Na⁺ stress</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSI light</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td>PSI light</td>
<td>2.7</td>
<td>1.5</td>
</tr>
<tr>
<td>Dark, with glucose I</td>
<td>2.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Dark, with glucose II</td>
<td>2.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

² This level was 2.3 times higher than that of control cells grown under PSI light in the experiments for CO₂ stress.

Basal levels, levels without stress, are expressed as values relative to the smallest under PSI light. Enhancing rates are ratios between levels with and without stress.
of enhancement was also similar in the two enhancements. Together with a close relationship, a parallel variation of the electron turnover capacity of the two terminals of ETS strongly indicates that (1) three stresses cause a variation of photosystem stoichiometry through a common regulatory mechanism. They further suggest that (2) the electron turnover capacity of the two terminals, PSI and Cyt c oxidase, is regulated under a common signal monitoring mechanism.

If the above is correct, effects of CO₂ and Na⁺ stresses can be explained by our work model for the regulation responding to the light stress (Fujita et al. 1994). Our model suggests that PSI/PSII stoichiometry is controlled by monitoring reduced level of Cyt b₅; higher reduced level induces higher stoichiometry (Murakami and Fujita 1991, 1993). When cells suffer with either stress, energy requirement may increase for exclusion of Na⁺ ion from cells (Molitor et al. 1986) or for incorporation of HCO₃⁻ ion into cells (cf. Kaplan et al. 1994). Such energy requirement may elevate the proportion of the cyclic electron flow to non-cyclic one in photosynthetic electron transport. Elevation of the cyclic electron flow flux back to plastoquinone (PQ) may increase the electron influx to the electron pool between the two photosystems and lead to elevation of the reduced level of Cyt b₅. In the dark, the respiratory electron flow flux may increase due to the extra energy requirement resulting in a higher reduced state of Cyt b₅ because respiratory electron transport in this organism appears to be limited at the step of Cyt c oxidase (Fujita et al. 1987). According to our work model, PSI formation is stimulated in proportion to the reduced level of this cytochrome. Increase in PSI abundance enhances the electron turnover capacity of PSI relative to that of PSII. Such a change balances the two photosystem actions in the electron transport of a new proportion of the cyclic to non-cyclic electron flow. In the dark, elevation of Cyt c oxidase activity may release the respiratory electron transport state from biassed state. The effect of Na⁺ stress disappeared upon light illumination (Table 3). The electron flow to O₂ driven by PSI possibly works off the biassed state.

General considerations—Some of the regulatory events in thylakoid system for oxygenic photosynthesis have been reported to depend on the redox state of ETS component(s) between the two photosystems such as PQ and/or Cyt b₅f. State transitions (cf. Allen 1992, Fujita et al. 1994), the regulation of photosystem stoichiometry (Fujita et al. 1994) and the regulation of cab gene expression (Escobas et al. 1995, Maxwell et al. 1995) are such examples. These events occur for maintaining the balance between the two photosystem actions, thus, the balance between the electron influx to and the efflux from the thylakoid electron transport. Although mechanisms hypothesized for respective events are not necessarily identical, the redox state of PQ and/or Cyt b₅f is possibly monitored under a common mechanism.

If it is correct, such a mechanism must play a central role in balancing the thylakoid electron transport. Variation of PSI/PSII stoichiometry may be an appearance of one of such regulations. The finding that Cyt c oxidase activity in thylakoids is also regulated suggests a possibility that the regulation of this type also occurs in respiratory ETS of heterotrophic organisms.

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References


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