Acclimation of the Photosynthetic Apparatus to Photosystem I or Photosystem II Light: Evidence from Quantum Yield Measurements and Fluorescence Spectroscopy of Cyanobacterial Cells

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Cells of the cyanobacterium *Synechococcus* 6301 were grown under illumination whose spectral composition favoured absorption either by the phycobilisome (PBS) light-harvesting antenna of photosystem II (PS II) or by the chlorophyll (Chl) *a* light-harvesting antenna of photosystem I (PS I). Cells grown under PS I-light developed relatively high PS II/PS I and PBS/Chl ratios. Cells grown under PS II-light developed relatively low PS II/PS I and PBS/Chl ratios. Thus, the primary difference between cells in the two acclimation states appeared to be the relative concentration of PBS-PS II and PS I complexes in the thylakoid membrane. Measurements of the quantum yield of oxygen evolution suggested a higher efficiency of cellular photosynthesis upon the adjustment of photosystem stoichiometry to a specific light condition. The quantum yield of oxygen evolution was nevertheless lower under PBS than Chl excitation, suggesting quenching of excitation energy in the photochemical apparatus of PS II in *Synechococcus* 6301. This phenomenon was more pronounced in the PS II-light than in the PS I-light grown cells. Room temperature and 77 K fluorescence emission spectroscopy indicated that excess excitation energy in the PBS was not transferred to PS I, suggesting the operation of a non-radiative and non-photochemical decay of excitation energy at the PBS-PS II complex. This non-photochemical quenching was specific to conditions where excitation of PS II occurred in excess of its capacity for useful photochemistry.

Introduction

The thylakoid membrane organization and photosystem stoichiometry in cyanobacteria are distinctly different from those of higher plant chloroplasts and green algae. In sun-adapted higher plant chloroplasts, like spinach and peas, the photosystem stoichiometry (PS II/PS I ratio) is approximately 1.8 [1–5], and the two photosystems are segregated in the grana partition regions (PS II) and stroma exposed lamellae (PS I) [6–8]. In cyanobacteria (and red algae), the photosystem stoichiometry is much lower than unity, with typical values of PS II/PS I = 0.5 [1, 9, 10]. Individual PS I complexes in cyanobacteria contain a light-harvesting antenna of 130 ± 10 Chl *a* molecules. In contrast, individual PS II complexes contain only 35–40 Chl *a* molecules in their light-harvesting antenna [10–13]. In spite of the lower stoichiometric quantity and the smaller Chl *a* antenna size of PS II in their thylakoid membrane, cyanobacteria maintain an overall balanced absorption of light and electron-transport between the two photosystems. This requirement is satisfied because light-harvesting by PS II is aided markedly by the auxiliary phycobilisome antenna. In *Synechococcus* 6301, the phycobilisome contains approximately 400 bilin molecules [11] which act as a light-harvesting antenna for PS II and thus help in balancing light-absorption by the two photosystems. Indeed, deconvolution of the absorbance spectra of *Synechococcus* 6301 cells into PBS-PS II and PS I components indicated about equal integrated absorbance of light by the two groups of complexes [14].

The organization of the thylakoid membrane in cyanobacteria, summarized above, is not static but dynamic in nature. It is now clear that the photosystem stoichiometry in the thylakoid membrane of oxygenic photosynthesis is adjusted and optimized in response to external conditions [3, 4]. In cyanobac-

Abbreviations: Chl, chlorophyll; PBS, phycobilisome; PS, photosystem; Io, incident light intensity; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

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teria, this was first indicated in the work of Kawamura *et al.* [9], as a response to changes in light-intensity during cell growth. Independent work from three laboratories [10, 15, 16] presented evidence of large variations in photosystem stoichiometry in response to a specific stimulus, *i.e.*, to the balance of light absorption by the two photosystems. Cell growth under light absorbed predominantly by Chl (PS I light) resulted in a bilin/Chl ratio of 1.3 and a PS II/PS I ratio of 0.7. Cell growth under light absorbed primarily by the PBS (PS II light) resulted in a decrease in each ratio, giving a bilin/Chl ratio of 0.42 and a PS II/PS I ratio of 0.27. It became evident that cyanobacteria (very much like higher plant chloroplasts) possess a feedback and control mechanism for the adjustment of photosystem stoichiometry [3]. Evidence was presented that a photosystem stoichiometry adjustment in a variety of photosynthetic tissues was necessary and sufficient to establish balanced integrated absorption of light by the two photoreactions in the thylakoid membrane [3, 16]. The only exception to this rule appeared in the case of *Synechococcus* 6301 grown under strict PBS light where a lowered photosystem stoichiometry (PS II/PS I = 0.27) was not quite sufficient to balance fully light absorption between the two photoreactions [16].

Changes in photosystem stoichiometry in response to light quality are now understood to be a compensation reaction in the thylakoid membrane, serving to correct uneven absorption of light by the two photosystems. In principle, balanced excitation energy distribution between PS I and PS II could also be achieved by regulation of spillover of excitation energy to PS I [17–19] or by an alteration in the optical absorption cross-section of PS II relative to that of PS I [20–22]. These alternative mechanisms for balancing the operation of the two photosystems may contribute to short-term adaptations described as state 1-state 2 transitions [23–25].

Here we describe evidence on long-term acclimation of the photosynthetic apparatus in the cyanobacterium *Synechococcus* 6301 by analysis of utilization of light energy under the two extreme acclimation conditions. Our results can be most easily understood in terms of alteration in photosystem stoichiometry (the PBS-PS II/PS I ratio) and therefore seem to exclude alteration of antenna size or of spillover in the developmental adaptation of *Synechococcus* 6301 to spectral quality of light.

Materials and Methods

Synechococcus 6301 *(Anacystis nidulans)* (UTEX 625) was grown photoautotrophically at 35 °C in medium C of Kratz and Myers [26]. Cells were grown to middle-log phase under continuous stirring and bubbling with a mixture of 5% CO_2 in N_2. Illumination was provided by a combination of tungsten incandescent lamps and a red cut-off filter (chromoid ruby 114 from Strand Lighting Ltd., Isleworth, Middlesex, U.K.) providing predominantly chlorophyll excitation (PS I-light). Alternatively, illumination was provided by cool-white fluorescent lamps and a golden-yellow cut-off filter (cinemoid orange 5 from Strand Lighting Ltd.), providing predominantly phycocyanin excitation (PS II-light). The spectral distribution of the light sources used in this work is shown in Fig. 1 (upper). The photon flux densities of the photosynthetic irradiation for the two types of culture were adjusted to sustain about equal rates of cell growth.

Oxygen evolution measurements of cells in their growth medium were obtained, immediately upon cell harvesting, with a Clark-type oxygen electrode (Hansatech Ltd., King's Lynn, U.K.). The reaction mixture was saturated in CO_2 (bubbled with a mixture of 5% CO_2 in N_2) and depleted in O_2. This condition helped to suppress both chlororespiration and photorespiration which might otherwise interfere with the measurement of the quantum yield. Actinic excitation of the samples in the oxygen electrode was provided by fiberoptic light-pipes. The quality of the actinic excitation was either predominantly Chl excitation (similar to PS I-light used for the cell growth) or predominantly PBS excitation (similar to the PS II-light used for the cell growth). Actinic illumination was provided by a high intensity tungsten filament lamp. PS I light and PS II light were defined by the same filters used for cell growth: in the case of PS II light an Ealing 660 nm short-wavelength band-pass filter was used to correct for the difference in spectral quality between the tungsten filament lamp and the fluorescent lamps used for cell growth. The unattenuated intensities (PAR) of the actinic beams at the oxygen electrode were 1000 and 1300 $\mu E \cdot m^{-2} \cdot s^{-1}$ for the PS I-light and for the PS II-light, respectively. Attenuation of the incident intensity was provided by neutral density filters.

Fluorescence induction transients were recorded with cells in a stirred 1 cm glass cuvette in the pres-

ence of 50 μM DCMU. Cells were adapted to state 1 by exposure to far-red light prior to adding the DCMU and recording the transient as described [22]. Actinic illumination was provided at about 570 nm (PBS excitation) at an intensity of 30 $\mu E \cdot m^{-2} \cdot s^{-1}$ with a combination of a Corning 4-96 broad-band filter and an Ealing 560 nm long-wavelength band-pass cut-off filter. Room temperature and liquid nitrogen temperature fluorescence emission and excitation spectra were recorded with a Perkin-Elmer LS-5 fluorescence scanning spectrometer. For 77 K spectra, suspensions of cells in their growth medium were driven to state 1 by 5 minutes' exposure to far-red light (709 nm) at an intensity of 300 $\mu E \cdot m^{-2} \cdot s^{-1}$ before being rapidly frozen in liquid nitrogen. Spectra were normalized to the phycocyanin fluorescence emission peak at 654 nm. For room-temperature spectra cells were maintained in state 1 by superimposing a continuous blue light (100 $\mu E \cdot m^{-2} \cdot s^{-1}$) defined by a combination of of Corning 4-96 and Corning 5-60 filters on the weak measuring beam. Spectra from different cell samples were normalized to the phycocyanin fluorescence emission peak at 650 nm: this normalization was performed when the cells were at F_o in state 2 (adapted to the weak measuring beam) because under these conditions the contribution of chlorophyll fluorescence at 650 nm was negligible. Spectra for cells at the F_m level of fluorescence were recorded in the presence of 50 μM DCMU. Cell concentration for all measurements was about 5 μM Chl *a*. Details of the methods measured were described in [22].

Results

Acclimation of cyanobacteria to PS I or PS II light is followed by pronounced shifts in the ratio phycobilin/Chl and in the PS II/PS I ratio in the thylakoid membrane. Fig. 1 (upper) shows the emission profile of broad-band light sources designed to sensitize predominantly the phycobilisome (PS II-light) or chlorophyll (PS I-light), *Synechococcus* 6301 cultures grown under these different light regimes show markedly different absorbance characteristics [27], Fig. 1 (lower) shows that PS I-light grown cells have a high A_{625}/A_{678} ratio (high PBS/Chl) whereas in PS II-light grown cells this ratio is significantly lower (Table I). The underlying cause of the shift in pigment ratios is substantially different concentrations of PBS-PS II and PS I complexes in the thylakoid

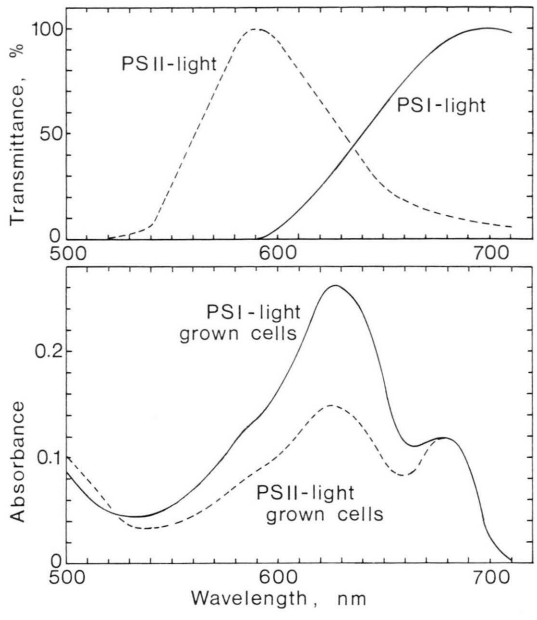


Fig. 1. (Upper) The wavelength dependent emission profile of the light sources designed to provide mainly phycobilisome excitation (PS II-light) or chlorophyll excitation (PS I-light). The transmittance spectra were normalized to 100% at the wavelength of maximum emission. (Lower) Absorbance spectra of *Synechococcus* 6301 cells acclimated to PS I-light (PS I-light grown cells) or to PS II-light (PS II-light grown cells). The absorbance spectra from the two cell types were normalized at the Chl maximum (678 nm). Note the dissimilar phycocyanin/chlorophyll ratio.

Table I. Thylakoid membrane properties in *Synechococcus* 6301 cells acclimated to PS I-light or PS II-light. Phycocyanin/Chl absorbance ratios were measured from the absorbance spectra of dilute cell suspensions after correction for scattering. The integrated absorbance of light σ was obtained as the integral of the quantity $A(\lambda)$, $T(\lambda)$, $d(\lambda)$, where $A(\lambda)$ is the absorbance spectrum of *Synechococcus* 6301 cells and $T(\lambda)$ is the transmittance profile of the respective light source (Fig. 1), σ values are given in arbitrary units. The relative quantum yield of photosynthesis θ was estimated from the apparent quantum yield (Fig. 3A and 3B) after correction for the integrated absorbance of light by each sample in each measurement. The quantum yield of PS I-light grown cells, measured under PS I illumination, was arbitrarily normalized to 1.0.

	PS I-light grown cells	PS II-light grown cells
A625/A678	2.2	1.26
σ(PS I-illumination)	730	550
θ(PS I-illumination)	1.0	0.75
σ(PS II-illumination)	935	575
θ(PS II-illumination)	0.30	0.50

membrane under the two light regimes [10, 16, 28]. The adjustment of photosystem stoichiometry is now understood to be a compensation type of response of the cell, designed to establish approximately equal absorption of light by PBS-PS II and PS I complexes in the thylakoid membrane under the two extreme light quality conditions [3, 16, 29]. It is assumed that such response helps the cells perform photosynthesis efficiently under the two extreme light conditions. The following results provide a comparison of the photosynthetic properties of cells acclimated under the two light regimes.

Rate of photosynthesis

To confirm that changes in the PBS/Chl ratio do not simply reflect an adjustment of individual PBS antenna size, we compared the variable fluorescence induction kinetics of DCMU poisoned *Synechococcus* 6301 cells, grown under PS I-light or under PS II-light conditions. The fluorescence induction kinetics of cells in the presence of DCMU provide a relative measure of the absorption cross-section of cells for the particular wavelength of excitation. Upon actinic illumination at 570 nm, the fluorescence induction kinetics will compare the relative absorption cross-section of the PBS in the two cell types. Fig. 2 compares the variable part of the fluorescence induction kinetics of cells grown under PS I or PS II-light. There is only a small difference in the fluorescence induction kinetics of the two samples, suggesting similar PBS-PS II antenna size in the two cell types.

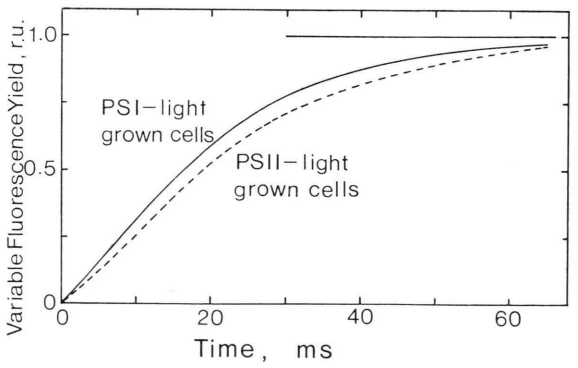


Fig. 2. Variable Chl *a* fluorescence induction kinetics of *Synechococcus* 6301 cells grown under PS I-illumination or under PS II-illumination. Cells were suspended in the presence of 50 μM DCMU. Actinic excitation was provided at about 570 nm.

This interpretation is consistent with earlier measurements [16] suggesting a PS stoichiometry adjustment rather than antenna size adjustment as the underlying cause of the different absorption spectra such as those seen in Fig. 1 (lower).

The fitness of the two cell types for photosynthesis was evaluated from the light-saturation curves of photosynthesis, measured both under PS I-light and PS II-light conditions. Fig. 3A compares the rate of photosynthesis in PS I-light grown cells (solid line) and in PS II-light grown cells (dashed line) as a function of the incident light intensity (PS I-light as defined in Fig. 1, upper). In this presentation, the rate of photosynthesis was measured on a per Chl basis. It

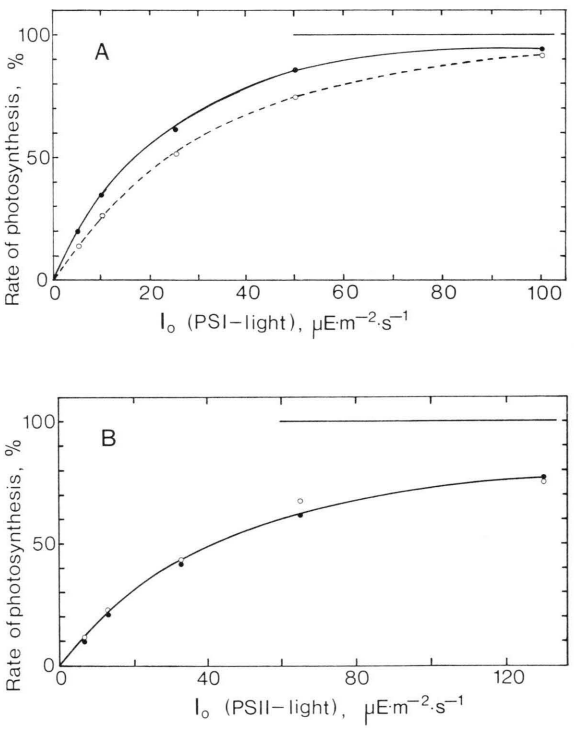


Fig. 3. Light-saturation curves of photosynthesis (measured as the rate of oxygen evolution) in *Synechococcus* 6301 acclimated to PS I-light (solid line/circles) or to PS II-light (dashed line/open circles). A. Actinic illumination predominantly absorbed by Chl (PS I-light, as defined in Fig. 1) was attenuated by neutral density filters. The intensity of the unattenuated beam was 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$. B. Actinic illumination predominantly absorbed by the phycobilisome (PS II-light, as defined in Fig. 1) was used. The intensity of the unattenuated beam was 1300 $\mu E \cdot m^{-2} \cdot s^{-1}$. The light-saturated rate of photosynthesis was 300 (± 30) μM oxygen per μM Chl per h, and it was independent of cell culture and quality of actinic illumination.

was corrected for the dark respiration (about 20% of the light-saturated rate). The light-saturated rates of photosynthesis were similar in the two cell types with typical values (corresponding to 100% in Fig. 3) of about 300 μM oxygen per μM Chl per h.

The results of Fig. 3A show that, under PS I-illumination conditions, the apparent quantum yield of photosynthesis (slope of the curve at Io = 0 $\mu E \cdot m^{-2} \cdot s^{-1}$) is greater for the PS I-light grown cells (solid line) than for the PS II-light grown cells (dashed line). From the results of Fig. 3A we estimated the apparent quantum yield of photosynthesis for the PS II-light grown cells to be only about 56% of that for the PS I-light grown cells. However, the rate of photosynthesis in Fig. 3A is plotted as a function of the incident rather than the absorbed light intensity. A more meaningful comparison of the quantum yield of photosynthesis between the two samples can be obtained only upon correction for the absorbed intensity of PS I-light which is clearly different in the two cell types because of their different absorbance spectra (Fig. 1, lower). Fig. 4A compares the effective absorbance of PS I-light by PS I-light grown cells (solid line) and by PS II-light grown cells. (The effective absorbance spectra of Fig. 4A were obtained as the product A(λ), T(λ) where A(λ) is the absorbance spectrum of each cell type and T(λ) is the emission profile of the PS I-light source.) The integrated absorbance of PS I-light, σ(PS I-light), by the two cell types was obtained directly from the results of Fig. 4A upon integration of the effective absorbance spectra. The respective values of σ(PS I-light) were 730 and 550 arbitrary units for PS I-light grown cells and for PS II-light grown cells, respectively (Table I). It is evident from these results that PS I-light grown cells will absorb about 32% more light than their PS II-light grown counterparts (730 *versus* 550 relative units of integrated absorbance). Corrected for this difference in integrated absorbance of light between the two cell types, the quantum yield of photosynthesis for the PS II-light grown cells is about 0.75 of that for the PS I-light grown cells (θ(PS I-illumination), Table I). The results clearly suggest that acclimation of *Synechococcus* 6301 to PS I-light enables the cells to perform photosynthesis more efficiently under this light regime.

Fig. 3B compares the light saturation curves of photosynthesis of PS I-light grown cells (solid circles) and PS II-light grown cells (open circles) as a function of incident (PS II-light) intensity. Under

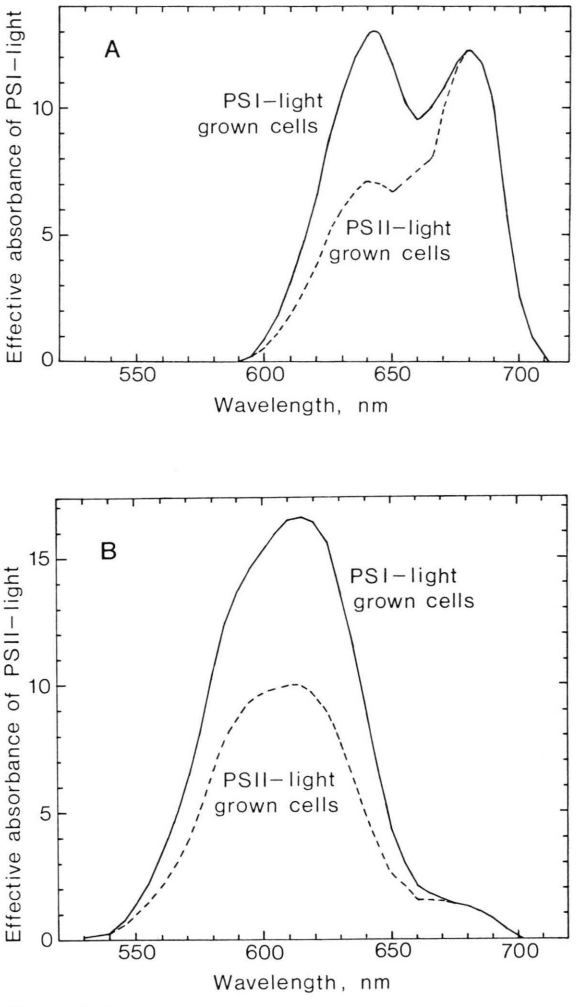


Fig. 4. (A) Effective absorbance of PS I-light by *Synechococcus* 6301 cells grown under PS I-illumination (solid line) or under PS II-illumination conditions (dashed line). The effective absorbance spectra were obtained upon multiplication of the absorbance spectrum of each cell type (Fig. 1, lower) with the transmittance spectrum of the PS I-light emitting source. (B) Effective absorbance of PS II-light by *Synechococcus* 6301 cells grown under PS I-illumination (solid line) or under PS II-illumination conditions (dashed line). The effective absorbance spectra were obtained upon multiplication of the absorbance spectrum of each cell type with the transmittance spectrum of the PS II-light emitting source. Note the greater relative absorbed intensity of light by the PS I-light grown cells.

these conditions, the apparent quantum yield of photosynthesis was similar for the two cell types and apparently lower than that measured with PS I-light (Fig. 3A). Fig. 4B shows the effective absorbance of PS II-light by PS I-light grown cells (solid line) and

by PS II-light grown cells (dashed line). It is evident from the effective absorbance spectra of Fig. 4B that most of the PS II-light is absorbed by the PBS in the two cell cultures. With incident PS II-light (Fig. 4B), the integrated absorbance σ(PS II-light) of the two cell types was 935 and 575 relative units for PS I-light grown cells and for PS II-light grown cells, respectively (Table I). From the results of Fig. 3B and from the integrated absorbance of PS II-light by the two cell types we estimated a relative quantum yield of 0.30 for the PS I-light grown cells and of about 0.50 for the PS II-light grown cells (Table I). The results suggest that, under PS II-illumination, PS II-light grown cells ($\theta = 0.50$) perform photochemistry more efficiently than PS I-light grown cells ($\theta = 0.30$). In both cases, however, PS II-illumination (PBS excitation) is much less efficient at driving photosynthesis than broad band Chl light. It may be concluded that a substantial fraction of excitation energy (PBS excitation in this case) is not converted to useful photochemistry but is lost *via* a non-photochemical quenching process. Further evidence for the operation of a non-photochemical quenching in *Synechococcus* 6301 is described below.

Room temperature fluorescence spectroscopy

The functional properties of PS I-light and PS II-light grown cells were probed further with fluorescence spectroscopy. Fig. 5 (upper) shows fluorescence emission spectra from the two cell types, obtained upon excitation of the cells at 580 nm. The cells were adapted to state 1 and spectra were normalized to phycocyanin emission as described in Materials and Methods. The main Chl emission peak is observed at 685 nm. Fig. 5 (lower) shows the same spectra upon subtraction of the phycocyanin emission (trace marked with solid squares in Fig. 5, upper). The yield of the fluorescence emission at the F_o level was similar for the two cell types. At the F_{max} level, however, when all PS II reaction centers are closed, there was a consistent difference in the fluorescence yield between PS I-light grown cells (solid line) and PS II-light grown cells (dashed line). The fluorescence yield of the latter was only about 60% of that in the former, suggesting that, under reducing conditions, 40% of the excitation absorbed by PS II in the PS II-light grown cells was quenched. This interpretation received further support from fluorescence excitation spectra for the variable component of PS II fluorescence (Fig. 6). These spectra show the relative contributions of absorption by chlorophyll *a* at 435 nm and of absorption by the phycobilisome at 550–660 nm to the variable component of fluorescence emission at 685 nm, which originates exclusively from PS II. In PS II-light

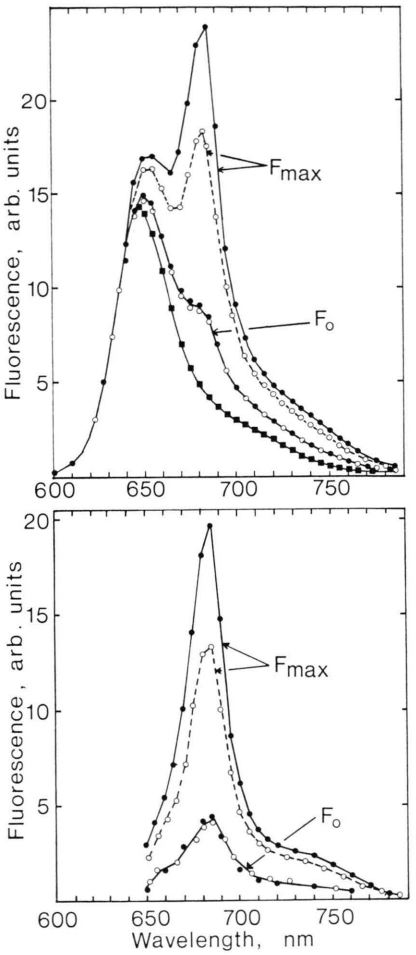


Fig. 5. Room temperature Chl *a* fluorescence emission spectra of intact *Synechococcus* 6301 cells excited at 580 nm. Cells were grown under PS I-light (solid line/circles) or PS II-light conditions (dashed line/open circles). Spectra for different samples were normalized to the phycocyanin fluorescence emission as described in Materials and Methods. (Upper) Fluorescence spectra of the F_o and F_{max} emission were measured separately. Also shown is the fluorescence emission spectrum of phycocyanin (squares). Note the 685 nm emission from PS II and the significantly lowered amplitude of this emission in PS II-light grown cells (dashed line). (Lower) The same spectra after subtraction of the phycocyanin emission.

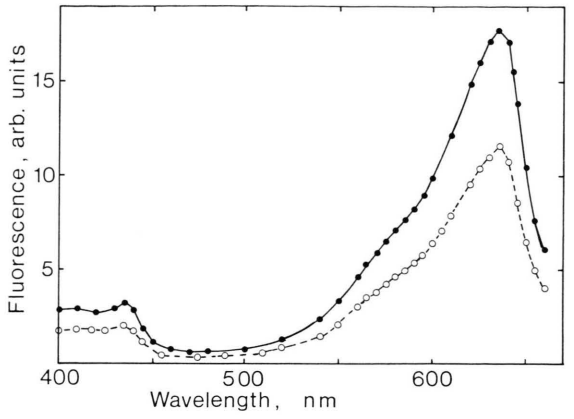
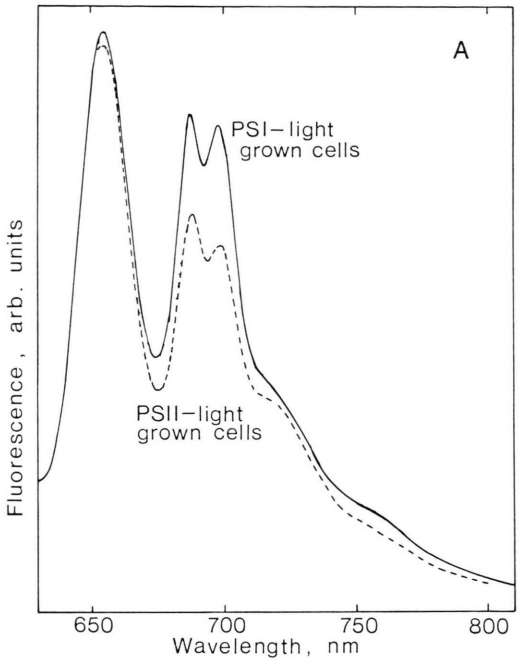


Fig. 6. Room temperature excitation spectra for the variable component of PS II fluorescence emission in *Synechococcus* 6301 cells. The fluorescence emission at the F_{max} level and the F_o level was measured at 685 nm. The excitation spectra for F_v were then obtained by subtracting the F_o spectrum from the F_{max} spectrum. Since the variable component of fluorescence originates exclusively from the Chl of PS II, these difference spectra contain no contribution of phycocyanin fluorescence emission at 685 nm. Cells were grown under PS I-illumination (solid line) or PS II-illumination (dashed line). Note the lower fluorescence emission of PS II-light grown cells, elicited upon phycocyanin and chlorophyll excitation.



grown cells the amplitude of the excitation spectrum (relative to the phycocyanin fluorescence emission) is reduced by about 40% but the shape of the spectrum and the relative amplitudes of the Chl *a* and phycobilisome peaks are not significantly changed. This indicates that chlorophyll-absorbed excitation and phycobilisome-absorbed excitation by PS II are both quenched with similar efficiency in the PS II-light grown cells, which suggests a quenching mechanism operating at the level of the chlorophyll pigment bed of PS II. These observations corroborate the results of Fig. 3B and of the quantum yield measurements under PS II-light, θ(PS II-light). Furthermore, they are consistent with earlier measurements by Manodori and Melis [16] and suggest the presence of a non-photochemical quenching process under conditions of PS II overexcitation.

Low temperature fluorescence spectroscopy

Additional information was derived from low temperature fluorescence spectroscopy of PS I-light and PS II-light grown cells. Fig. 7A shows fluores-

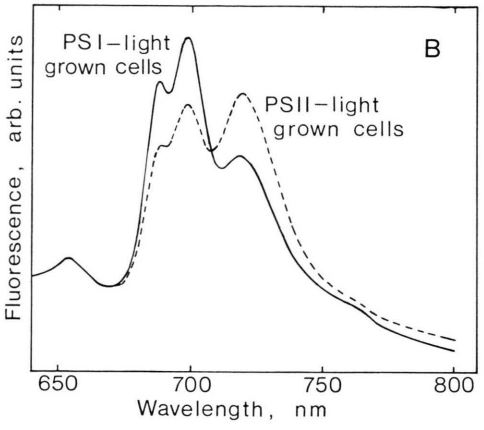


Fig. 7. Low temperature (77 K) fluorescence emission spectra of *Synechococcus* 6301 upon excitation at 580 nm (A) and at 435 nm (B). The spectra were normalized to the phycocyanin emission at 650 nm. The bands at 685 nm and 695 nm are attributed to emission of light by the Chl pigments of PS II whereas the band at 720 nm is due to fluorescence emission from the Chl pigments of PS I. Note the substantially lower PS II fluorescence emission in the PS II-light grown cells in (A), and the dissimilar PS II and PS I fluorescence yields in PS I-light and PS II-light grown cells (B).

cence emission spectra at 77 K of PS I-light and PS II-light grown cells upon excitation at 580 nm. The spectra were normalized to the emission of phycocyanin at 650 nm. One distinct difference between the two cell types is the lowered fluorescence yield at 685 nm and 695 nm (PS II fluorescence) in the PS II-light grown cells. This is in agreement with the results of room temperature fluorescence emission spectra shown in Fig. 5. The fluorescence emission of PS I at 720 nm is discerned only as a low amplitude shoulder in the emission spectra. The low amplitude emission at 720 nm could be explained as originating upon direct excitation of the PS I Chl at 580 nm. It is somewhat more pronounced for the PS II-light grown cells because these cells have a lower phycocyanin/Chl ratio, thereby allowing for greater direct absorption of 580 nm light by Chl relative to phycocyanin. In either case, it would appear that PBS excitation is transferred mainly to PS II centers and that it does not reach PS I in appreciable quantities. Moreover, the lower fluorescence yield at 685 nm and 695 nm in the PS II-light grown cells is unlikely to be attributable to a spillover of excitation from PS II to PS I because a corresponding increase in the 720 nm emission is not observed.

Fig. 7B shows fluorescence emission spectra at 77 K of PS I-light and PS II-light grown cells upon excitation at 435 nm. A distinct feature here is the greater fluorescence yield of PS II (685 and 695 nm) in the PS I-light grown cells, and the greater fluorescence yield of PS I (720 nm) in the PS II-light grown cells. Since excitation at 435 nm is absorbed predominantly by Chl, the increased 720 nm fluorescence in PS II-light grown cells simply reflects the enhanced PS I concentration in the PS II-light grown cells. Since the spectra were normalized to the 650 nm fluorescence emission, the lowered 685 nm and 695 nm fluorescence in PS II-light grown cells indicates lowered PS II fluorescence emission relative to phycocyanin. Since the fluorescence induction kinetics (Fig. 3A) indicate little change in the ratio of PS II to PBS, the lowered PS II emission must result from a non-photochemical quenching process similar to that observed with PBS-absorbed light in Fig. 6. This conclusion supports the interpretation of the room-temperature fluorescence excitation spectra (Fig. 6) which indicate that a non-photochemical quenching process operates under conditions of PBS-PS II over-excitation in *Synechococcus* 6301.

Discussion

The results from this work clearly suggest that changes in photosystem stoichiometry in the thylakoid membrane of cyanobacteria represent adjustment and optimization of the electron transport process enabling the cells to maintain an efficient quantum yield of photosynthesis. In summary, *Synechococcus* 6301 grown under predominantly PS I-illumination (mostly Chl excitation) develop a high phycocyanin/Chl ratio and a relatively high PS II/PS I reaction center ratio. Conversely, *Synechococcus* 6301 grown under predominantly PS II-illumination (mostly PBS excitation) develop a lower phycocyanin/Chl ratio and a lower PS II/PS I reaction center ratio. Cells having a relatively high PS II/PS I reaction center ratio perform photosynthesis more efficiently under PS I-light than cells with a lower PS II/PS I reaction center ratio. Cells having a relatively low PS II/PS I reaction center ratio perform photosynthesis more efficiently under PS II-light than cells with a higher PS II/PS I ratio. Thus, the adjustment in photosystem stoichiometry in the thylakoid membrane of *Synechococcus* 6301 is a necessary response designed to maintain a high photochemical efficiency in the electron transport process.

The findings from this work have important implications for plant growth and productivity under physiological conditions. In the aquatic environment, where cyanobacteria live, there are pronounced shifts in light quality (and light intensity) with distance from the surface. Cells growing close to the surface of the water will experience a light environment that favors greater absorption of irradiation by the phycobilisome. Away from the surface, however, the quality of light shifts toward the blue which is much better suited for direct absorption by Chl [30]. In an aquatic ecosystem, the light quality also depends strongly on the presence of green algae which absorb light in the blue and red regions of the spectrum (Chl absorption) thereby allowing selective transmission of light in the green and yellow regions (PBS region). Hence, the adaptability of the photosystem stoichiometry in the thylakoid membrane enables photosynthetic cells to adjust and optimize function in a broad variety of light environments. This will confer a selective advantage upon cells possessing such a mechanism for adaptation.

The molecular and biochemical basis of the feedback control mechanism for the regulation of photo-

system stoichiometry in the thylakoid membrane is not understood [3]. Preliminary measurements in this laboratory have shown that cells acclimate from one extreme PS II/PS I ratio to the other with a half-time of about 20 h. This suggests the involvement of both biosynthetic and degradative reactions in the process of thylakoid membrane change. The results from this work also suggest that there are limits to the ability of the cell to respond to an extreme imbalance in light absorption by PS II and PS I. For example, under the PS II-light growth conditions used in this work, the adjustment in photosystem stoichiometry was necessary but not quite sufficient to balance fully the rate of light absorption by the two photoreactions. It was estimated that under our conditions, and in spite of the photosystem stoichiometry adjustment, PS II received about 40–50% more excitation (*via* the PBS) than PS I. This surplus PS II excitation clearly was not transferred to PS I since it could not be detected either in the quantum yield of oxygen evolution or as increased low temperature fluorescence emission from PS I (Fig. 7A). We suggest that the surplus PS II excitation energy is dissipated in a non-photochemical energy-dependent quenching process, analogous to that described by several authors for higher plant chloroplasts [31–37]. The operation of this non-photochemical quenching is manifested in our work as a quenching of PS II fluorescence, occurring both at room temperature and liquid nitrogen temperature, in cells grown under PS II-light. The non-photochemical quenching also contributes to the lower quantum yield of photosynthesis of PS II-light grown cells ($\theta = 0.50$) relative to that of PS I-light grown cells ($\theta = 1.0$, Table I). Thus, the results and conclusions from this work add new information on this topic and further corroborate earlier work by Manodori and Melis [16] and by Murakami and Fujita [29]. One important difference with the work of the latter authors is that in the present study no significant difference was found between the light-saturated rates of the two cell types when measured under PS I or PS II actinic illumination [29].

Ley and Butler [17] concluded from studies of chromatic adaptation in the red alga *Porphyridium cruentum* that adaptation could be explained by changes in spillover of excitation energy from PS II to PS I, by altered chlorophyll distribution between PS II and PS I, and by altered antenna size. Although there may be important experimental differences between the present study and that of Ley and Butler, it is possible that their data [17] is also consistent with alteration in photosystem stoichiometry, an alternative explanation apparently not considered at that time.

Different cyanobacterial and red algal species may possess additional adaptive mechanisms that might regulate excitation energy distribution. Complementary chromatic adaptation, for example, is found in many cyanobacterial species [38] and involves changes in the relative amounts of phycoerythrin and phycocyanin in the phycobilisome [38, 39]. Complementary chromatic adaptation alone will effectively produce alterations in the PBS antenna size for specific wavelengths of light. It is interesting to consider the possibility that complementary chromatic adaptation may be superimposed on changes in photosystem stoichiometry.

One potential use of *Synechococcus* 6301 cells grown under PS I-light and PS II-light conditions is in the study of state transitions in this organism. It is reasoned that PS I-light grown cells (high phycocyanin/Chl and PS II/PS I ratios) will readily attain a state 2 condition upon PBS excitation. Conversely, PS II-light grown cells (lower phycocyanin/Chl and lower PS II/PS I ratios) will promptly attain a state 1 condition upon Chl excitation, thereby facilitating a better characterization of the function of the photochemical apparatus under the two light-state conditions. The possibility also exists that acclimation of the photosynthetic apparatus involves a redox-controlled protein phosphorylation reaction which is implicated in state transitions in cyanobacteria [20] as well as in green plants [40]. Control of photosystem stoichiometry by the redox state of the plastoquinone pool has been implicated in the identification of electron transport imbalance between the two photosystems [3, 41]. These and related problems are currently under investigation using the experimental system described here.

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