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Growth under Red Light Enhances Photosystem II Relative to Photosystem I and Phycobilisomes in the Red Alga *Porphyridium cruentum*[1]

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ABSTRACT

Acclimation of the photosynthetic apparatus to light absorbed primarily by photosystem I (PSI) or by photosystem II (PSII) was studied in the unicellular red alga *Porphyridium cruentum* (ATCC 50161). Cultures grown under green light of 15 microeinsteins per square meter per second (PSII light; absorbed predominantly by the phycobilisomes) exhibited a PSII/PSI ratio of 0.26 ± 0.05. Under red light (PSI light; absorbed primarily by chlorophyll) of comparable quantum flux, cells contained nearly five times as many PSII per PSI (1.21 ± 0.10), and three times as many PSII per cell. About 12% of the chlorophyll was attributed to PSII in green light, 22% in white light, and 39% in red light-grown cultures. Chlorophyll antenna sizes appeared to remain constant at about 75 chlorophyll per PSII and 140 per PSI. Spectral quality had little effect on cell content or composition of the phycobilisomes, thus the number of PSII per phycobilisome was substantially greater in red light-grown cultures (4.2 ± 0.6) than in those grown under green (1.6 ± 0.3) or white light (2.9 ± 0.1). Total photosystems (PSI + PSII) per phycobilisome remained at about eight in each case. Carotenoid content and composition was little affected by the spectral composition of the growth light. Zeaxanthin comprised more than 50% (mole/mole), β-carotene about 40%, and cryptoxanthin about 4% of the carotenoid pigment. Despite marked changes in the light-harvesting apparatus, red and green light-grown cultures have generation times equal to that of cultures grown under white light of only one-third the quantum flux.

In photosynthetic organisms which contain PBsomes,[2] PSI and PSII have very different, almost complementary, action spectra. Most of the Chl *a* is associated with the light-harvesting antenna system of PSI (10, 11, 21, 23, 26, 29, 37). The green and orange light-absorbing phycobiliproteins appear to be associated with and initially transfer absorbed light energy predominantly to PSII (5, 21, 29, 37).

Acclimation of PBsome-containing organisms to light which is preferentially absorbed by the antennae of either PSI or PSII has been studied in several species of cyanobacteria (11–13, 24, 25, 29) and in a few red algae (13, 21). An increase in the ratio of PSII/PSI for cells grown under PSI light and a decrease for cells grown under PSII light is typically observed (11–13, 24, 25, 29). Unlike in higher plants and green algae with their accessory light-harvesting Chl *a*/*b* proteins, the Chl antenna sizes of the photosystems in cyanobacteria and red algae are relatively small (10, 11, 22, 23, 26, 29) and, in cyanobacteria at least, they appear to be invariant (11, 25, 29). The stoichiometry of PSII and PBsomes is also regarded as fixed and unaffected by changes in the spectral composition of the growth light both in cyanobacteria and in red algae (11, 13, 24, 25, 30).

The red alga *Porphyridium cruentum* has been the subject of a number of studies on the effects of light intensity (6 and references therein) and light wavelength (2, 3, 13, 21, 30) on photosynthesis and the photosynthetic light-harvesting apparatus. In a previous study we showed that the relative numbers of PSI, PSII, and PBsomes in cells of *P. cruentum* remain relatively constant irrespective of light intensity over nearly the entire growth range for this red alga (6–280 $\mu E \cdot m^{-2} \cdot s^{-1}$; 6). This is not the case when the wavelength composition of the growth light is varied. Fujita *et al.* (13) reported a twofold increase in the ratio PSII/PSI for cultures of *P. cruentum* grown under PSI light (RL) compared to those grown under PSII light (orange light), but concluded that PBsome size and the ratio PSII/PBsome did not change significantly (13, 30). Data from a study by Ley and Butler on a different isolate of *P. cruentum* (21), however, is suggestive of drastic changes in PSII-PBsome stoichiometry (see "Discussion").

In this paper we reexamine acclimation of the photosynthetic light-harvesting apparatus of *P. cruentum* to growth under PSI light or PSII light. Specifically, we report on changes in the cell content and stoichiometry of PSI, PSII, and PBsomes, and in the pigments which comprise these supramolecular complexes. We conclude that the ratio of PSII to PBsomes is not fixed but varies with the wavelength composition of the growth light in a manner which is expected to be advantageous to the organism. We also confirm that PBsome composition is unaffected by light quality, *i.e.* that 'complementary chromatic adaptation' does not occur in this organism.

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[2] Abbreviations: PBsome, phycobilisome; APC, allophycocyanin; DCBQ, 2,6-dichloro-*p*-benzoquinone; GL, green light; P_{700}, reaction center of PSI; PC, phycocyanin; PE, phycoerythrin; Q_A, primary quinone acceptor of PSII; RL, red light; WL, white light.

MATERIALS AND METHODS

Cell Culture

Cultures of *Porphyridium cruentum* (ATCC 50161) were grown as described previously (6). Briefly, 1 L batch cultures were grown on a rotary platform shaker at 80 cycles/min at a constant temperature of 18°C, and bubbled with 5% CO_2 in air. Continuous light was provided by Sylvania VHO daylight fluorescent tubes filtered by appropriately colored plastic sheets for RL (Roscolux No. 27; medium red; obtained from Kinetic Artistry, Takoma Park, MD) and GL (P-40; dark green, from Gelatine Products Company, Glen Cove, NY). The red and green light fields were chosen to specifically excite either Chl *a* (RL) or phycobiliproteins (GL), insofar as possible. We considered it of critical importance that only actively dividing cultures be used for a study of acclimation. Growth of *P. cruentum* under longer wavelength RL (660–700 nm), absorbed primarily by the Chl antenna of PSI, was considered insufficient (less than one generation in 6 weeks). Therefore, the RL field used in these experiments was designed to provide enough photons of shorter wavelengths (*e.g.* 600–660 nm) to allow a reasonable rate of growth. The spectra of the green and red light fields used for cell culture are shown in Figure 1. The quantum flux between 400 and 700 nm was about 15 $\mu E \cdot m^{-2} \cdot s^{-1}$ for each light field. Stock cultures were routinely maintained under WL of 35 $\mu E \cdot m^{-2} \cdot s^{-1}$, but were grown under red or green light for 4 or 5 generations over the course of about 1 month before use as inocula for the experimental cultures.

Isolation of Thylakoid Membranes

All cultures were harvested while in the exponential phase of growth between 3 and 4 × 10^6 cells/mL. Cell harvest and subsequent procedures were carried out under normal room lights at 0 to 4°C as described previously (6). Aliquots of cells, broken cells, and thylakoids were stored at −80°C until used.

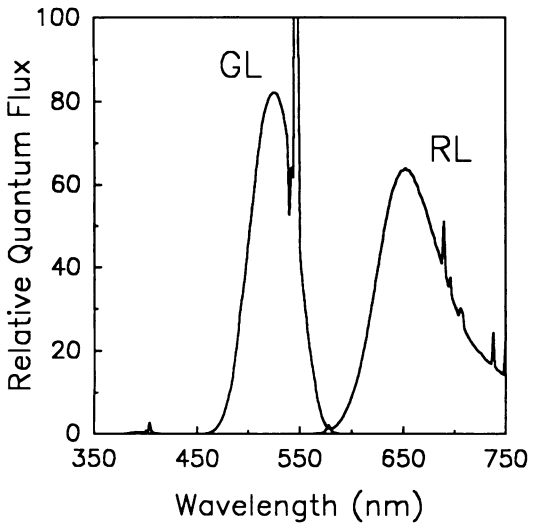


Figure 1. Spectra of red and green light fields used for culture of *P. cruentum*. Photon flux densities between 400 and 700 nm were about 15 $\mu E \cdot m^{-2} \cdot s^{-1}$ for each.

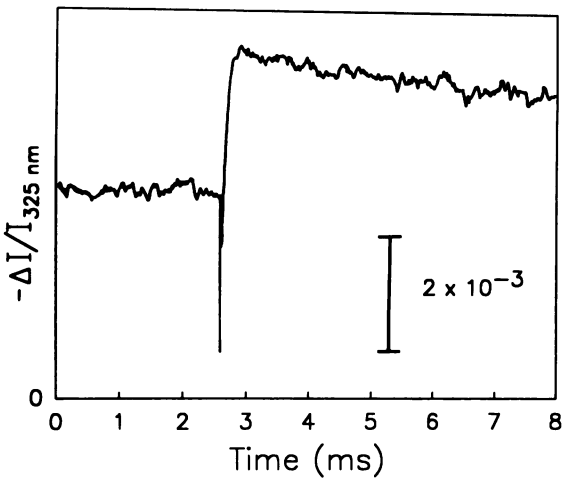


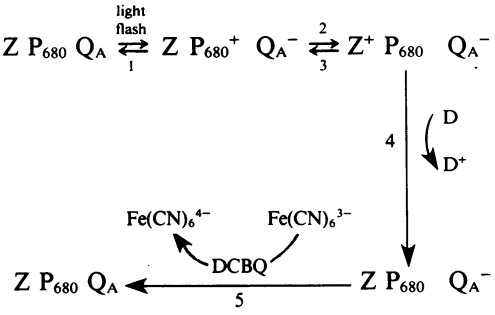
Figure 2. Flash-induced absorption change at 325 nm in thylakoid membranes of *P. cruentum* cultures grown under RL. The downward spike at 2.7 ms is from electronic interference due to the firing of the flash lamp. The electron acceptor system consisted of 25 μM 2,6-dichloro-*p*-benzoquinone and 500 μM potassium ferricyanide. The sample contained 25 μg Chl/mL. Multiple saturating flashes were given at 2 Hz. The trace shown is the average of 250 flashes. The electronic rise time for this measurement was 100 μs.

Pigment and Reaction Center Determinations

Pigments were quantified by absorption spectroscopy of whole cell extracts (Chl in *N,N'*-dimethylformamide according to Moran [27]; PE in 50 mM sodium phosphate buffer at pH 7.0 according to Gantt and Lipschultz [14]), by rocket immunoelectrophoresis (for stoichiometry of PE, PC, and APC), and by HPLC of acetone extracts of thylakoids and broken cells (Chl and carotenoids); all as described in a previous publication (6). PBsome numbers were calculated from the spectroscopic PE data by assuming 60 PE per PBsome in WL (see 6), but only 57.6 in RL, and 58.2 in GL-grown cells (based on the PE/APC ratios in Table III).

The concentration of P_{700} and Q_A were determined in thylakoid membranes by the light-induced absorption changes at 701 and 325 nm, respectively. Attempts to also quantify PSII by atrazine binding were unsuccessful because of a relatively low binding affinity of this herbicide for thylakoid membranes of *P. cruentum* (data not shown). The experimental protocol for the P_{700} measurement was the same as previously described (6, 17). For the measurement of Q_A, the multiple flash technique of Dennenberg *et al.* (8) was used. Steady state flashes of 0.08 $J \cdot cm^{-2} \cdot flash^{-1}$ were provided by two EG&G FX-200 flash lamps which were positioned on opposite sides of the cuvette. The flashes were given at 2 Hz, and absorption changes from 250 flashes were averaged. The electron acceptor couple was 500 μM potassium ferricyanide and 25 μM 2,6-dichloro-*p*-benzoquinone. Corrections for particle flattening were determined by the pigment extraction method of Pulles *et al.* (31).

The flash-induced absorption change at 325 nm (Fig. 2) corresponds to the formation of the species Q_A^-. In a sample that evolves oxygen, the reduction of Q_A results from electron flow through PSII with water as the source of the electrons. This 'normal' type of electron flow, however, did not take place in thylakoids of *P. cruentum* since it was found that they do not evolve oxygen (data not shown). What then is the source of electrons for the reduction of Q_A so clearly indicated by the absorption change shown in Figure 2? We suggest the following reaction scheme:



where P_{680} is the reaction center Chl of PSII, Z is the primary electron donor to P_{680}, Q_A is the primary electron acceptor of PSII, and D is an unidentified electron donor to Z. In this scheme, Q_A^- is oxidized either by charge recombination or by ferricyanide. Charge recombination, $Z^+ Q_A^- \rightarrow Z Q_A$, takes place via reactions 3 and 1 in the above scheme. If oxidation of Q_A^- is by ferricyanide mediated by DCBQ (reaction 5), then a source of electrons for reduction of Z^+ is also required. These are provided in our scheme by the hypothetical donor D via reaction 4. We measured the reduction of ferricyanide by its absorption change at 420 nm under the same conditions used to determine formation of Q_A^-. The results indicate that ferricyanide-mediated oxidation of Q_A^- can only account for about 10% of the Q_A^- recovery (data not shown). Recombination of charge, therefore, apparently accounts for 90% of the Q_A^- oxidation and Z^+ reduction between flashes. The remaining 10% of Z^+ reduction is provided for by donor D. A high percentage of recombination recovery of Q_A^- has also been suggested for thylakoids of spinach in which oxygen evolution was inhibited by washing of the membranes with Tris (2-amino-2-hydroxymethyl-1,3-propanediol) (39). What remains a mystery in our case is the source of electrons (the unidentified donor D in the reaction scheme) which replenish the reaction centers in those 10% of charge separations which do result in ferricyanide reduction.

The decay of the flash-induced absorption change at 325 nm in *P. cruentum*, corresponding to the oxidation of Q_A^-, has a lifetime of about 24 ms (Fig. 2). A comparable lifetime for Q_A^- (about 20 ms based on a reported rate of 50 recombinations per s) was observed for the Tris-washed thylakoid membranes of spinach (39). The 2 Hz flash rate which we used in our measurements is thus slow enough for complete recovery of the PSII reaction center between flashes.

RESULTS

Cell Growth

Cultures of *Porphyridium cruentum* grown under equivalent quantum flux of red or green light (15 $\mu E \cdot m^{-2} \cdot s^{-1}$ between 400 and 700 nm) exhibit comparable growth rates with generation times of about 1 week. Cells grown under WL are apparently much more efficient at collecting or utilizing the available photons, and achieve this growth rate under little more than one-third of the quantum flux (6 $\mu E \cdot m^{-2} \cdot s^{-1}$; 6). Previously published data for WL-grown cultures (6) will be compared with the data for cultures grown under red or green light.

Cell Pigment Content and Stoichiometry

The overall pigment content of *P. cruentum* cells differs little whether cultures are grown under low intensity red, green, or white light (Table I). Total amounts of Chl and carotenoids are comparable for RL and GL cells and are only a little higher for WL-grown cells. On an individual basis, the amounts of the three major carotenoids: zeaxanthin, β-carotene, and cryptoxanthin, which together account for 94 to 98% of the total carotenoid, are also present in comparable amounts. The total amount of unidentified carotenoids (comprised of six or seven minor components which are more polar than β-carotene and account, altogether, for only 2–6% of the total carotenoid) is substantially higher in RL compared to GL-grown cells, with WL-grown cells intermediate in amount. Several of these unidentified carotenoids were not detected in GL cells, but all were present in RL and WL cells (data not shown).

When the pigment data is presented on the basis of Chl *a*, the remarkable consistency of the pigment content becomes even more apparent (Table II). The carotenoid composition, relative to Chl *a*, is barely altered despite a fivefold change in the ratio of PSII to PSI (Table IV). The small increase in β-carotene as one proceeds from RL to WL to GL cells implies that this carotenoid is more prevalent (per Chl *a*) in PSI than in PSII. If all of the β-carotene is associated with the photosystems, an estimate of 6 to 10 β-carotene molecules per PSII and about 25 β-carotene per PSI is derived.

Phycoerythrin maintains a similar stoichiometry with Chl *a* whether *P. cruentum* is grown under RL or GL (Table II).

Table I. *Cell Content of Photosynthetic Pigments in P. cruentum*

Pigment	Growth Light		
	Green	Red	White
	mol/10¹⁷ cells		
Chl *a*	146 ± 18	164 ± 23	183±21
Unidentified Chl[a]	37 ± 18	7 ± 2	9 ± 1
Total Chl	183 ± 9	171 ± 26	192 ± 21
Zeaxanthin	33 ± 2	39 ± 6	42 ± 7
β-Carotene	29 ± 2	26 ± 4	32 ± 4
Cryptoxanthin	3.0 ± 0.3	2.5 ± 0.8	3.0 ± 0.5
Unidentified carotenoids	1.2 ± 0.1	4.1 ± 1.4	2.7 ± 0.3
Total carotenoids	65 ± 3	72 ± 11	80 ± 11
Phycoerythrin	10.3 ± 0.6	12.4 ± 1.5	11.8 ± 1.0

[a] Unidentified Chl refers to those peaks in HPLC elution profiles, other than Chl *a*, which absorb at both 650 and 442 nm. Data are mean ± SD of 3 to 5 independent experiments.

Table II. *Stoichiometry of Photosynthetic Pigments in P. cruentum*

Pigment	Growth Light		
	Green	Red	White
	mol/100 mol Chl a		
Zeaxanthin	23 ± 3	24 ± 1	23 ± 1
β-Carotene	20 ± 2	16 ± 1	18 ± 1
Cryptoxanthin	2.0 ± 0.2	1.5 ± 0.4	1.6 ± 0.1
Unidentified carotenoids	0.8 ± 0.1	2.5 ± 0.8	1.5 ± 0.3
Total carotenoids	45 ± 5	44 ± 2	44 ± 2
Phycoerythrin	5.7 ± 0.1	7.4 ± 0.4	6.1 ± 0.4

Data are mean ± SD of three to four independent experiments.

More importantly, the three major phycobiliproteins: PE, PC, and APC (14); are present in the same relative amounts whether in RL, GL, or WL cells (Table III). Absorption spectra of isolated PBsomes are comparable as well (data not shown). From these results we infer that PBsome size and composition are constant.

In acetone extracts of thylakoids from GL-grown cells as much as 30% of the absorbance at 650 nm (mean of about 20%; Table I) is due to compounds other than Chl *a*. These cells contain increased amounts of a component which elutes near the front (as expected for Chlide *a* or Pchlide *a*), and also have substantial amounts of other compounds which are not detected, or are present only in trace amounts, in cultures grown under low intensity RL (Fig. 3), or under a broad range of WL intensities (from 6–280 $\mu E \cdot m^{-2} \cdot s^{-1}$; data not shown). These compounds were not characterized further. The GL field that was used (Fig. 1) consists of wavelengths where the Pchl(ide) holochrome has little absorbance (33). The presence of significant quantities of RL-absorbing compounds other than Chl *a* in cultures grown under GL may reflect a specific wavelength requirement for Chl synthesis in *P. cruentum*.

Chl Antenna Sizes of PSI and PSII

The Chl antenna sizes of PSI and PSII were calculated by a method similar to that used by Fujita and Murakami (11). If one assumes that the number of antenna Chl per photosystem is constant, then the following equation will be valid:

$$Chl_t = (PSII \times Chl_{II}) + (PSI \times Chl_I)$$

rearranging:

$$Chl_t/PSI = Chl_{II} \times (PSII/PSI) + Chl_I \qquad (1)$$

where Chl_t is the total Chl in the sample, Chl_{II} is the number of Chl associated with each PSII, and Chl_I is the number of Chl associated with each PSI. Equation 1 predicts that a plot of Chl_t/PSI *versus* PSII/PSI will result in a straight line with a slope equal to Chl_{II} and an ordinate intercept equal to Chl_I. Such a plot, using data from Table IV and from an earlier publication (6), is shown in Figure 4A. Data points for cultures of *P. cruentum* grown under RL and GL of 15 $\mu E \cdot m^{-2} \cdot s^{-1}$, and WL of 6 and 35 $\mu E \cdot m^{-2} \cdot s^{-1}$, all fall on the same straight line plot. For these samples then, Chl_I appears to be constant and equal to about 140 Chl. A complementary plot shown in Figure 4B indicates that Chl_{II} is constant and equal to about 75. For cultures grown under 180 $\mu E \cdot m^{-2} \cdot s^{-1}$ (H) or 280 $\mu E \cdot m^{-2} \cdot s^{-1}$ (V) of continuous WL irradiance, the data points fall below the straight line plots of Figure 4. These cultures have 12% (H) and 18% (V) less Chl than predicted from Equation 1. Such a reduction in Chl_I and/or Chl_{II} may be due to a photobleaching. These constant high photon fluxes are saturating (H) or slightly inhibitory (V) to the growth of *P. cruentum* under our conditions (6).

Given the *y*-intercept estimates for Chl_I and Chl_{II} (Fig. 4) we calculate that, even in RL-grown cultures, most of the Chl is associated with PSI. PSII accounts for about 39% of the Chl in RL, 22% in WL (of 6 $\mu E \cdot m^{-2} \cdot s^{-1}$), and 12% in GL-grown cultures of *P. cruentum*.

Phycobilisome and Reaction Center Content and Stoichiometry

Growth under RL or GL results in drastic changes in the cell content and stoichiometry of PSI, PSII, and PBsomes in *P. cruentum* compared to amounts of these components measured in cells grown under WL. PSII centers, measured as Q_A, are three times more numerous in RL cells than in GL cells (Table IV). Conversely, PSI centers in RL cells, measured as P_{700}, are only two-thirds as numerous as in GL or WL cells (Table IV). Cell content of PBsomes (Table IV) differs little, if at all, whether cells are grown under continuous low intensity red, green, or white light.

As a consequence of the differences in cell content, the ratio of PSII to PSI increases nearly fivefold from 0.26 in GL cells to 1.21 in the RL-grown cultures (Table IV). The number of PSII per PBsome also changes, with a value for Q_A/PBsome of about 1.6 in GL cells and 4.2 in RL cells (Table IV). The combined total of photosystems per PBsome ($[Q_A + P_{700}]$/PBsome), however, remained constant at about 8 regardless of the spectral composition of the growth light (Table IV; Fig. 5). We have previously reported that WL-grown cultures maintain constant ratios of about 2.9 Q_A/PBsome and 0.4 to 0.5 PSII/PSI when grown under a broad range of white light intensities (6–280 $\mu E \cdot m^{-2} \cdot s^{-1}$; 6).

DISCUSSION

Stoichiometry of PSI, PSII, and PBsomes

Previous work has shown that when cultures of *P. cruentum* are placed under light conditions which favor the absorption of photons by either the antenna of PSI or the antenna of

Table III. *Stoichiometry of Phycobiliproteins in Cells of P. cruentum*

Phycobiliprotein Ratio	Growth Light		
	Green	Red	White
	% of mean for WL		
PE/APC (mol/mol)	97 ± 6	96 ± 5	100 ± 3
PC/APC (mol/mol)	102 ± 4	101 ± 3	100 ± 1

Data are mean ± SD of three to four independent experiments. Phycobiliproteins were quantified by rocket immunoelectrophoresis. The molar ratio of pigments in WL-grown cultures was estimated to be about 60:12:8 (PE:PC:APC).

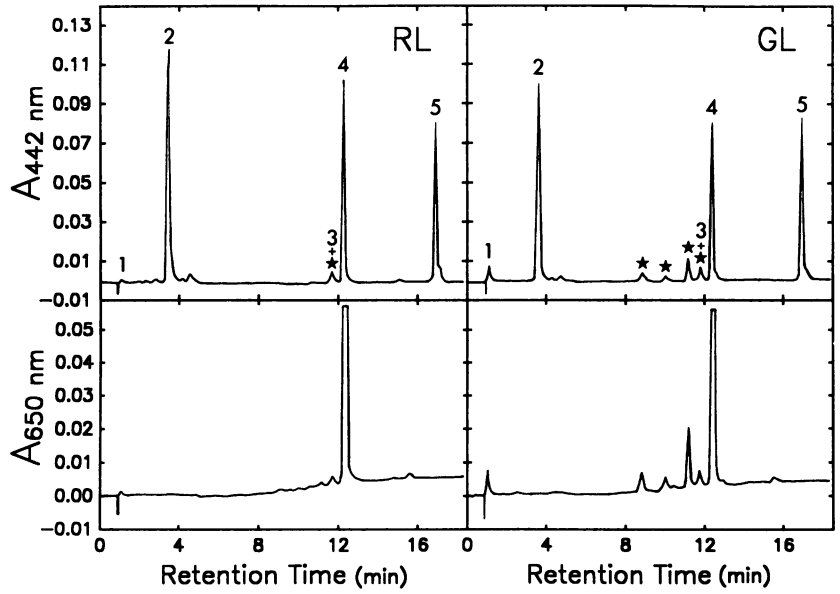


Figure 3. HPLC elution profiles of acetone-soluble pigments extracted from thylakoids of red and green light-grown cultures of *P. cruentum*. Absorbance was monitored at both 442 nm (top) and 650 nm (bottom) after injection of samples containing about 30 μg total Chl. Peak identifications: 1, Chlide *a*; 2, Zeaxanthin; 3, Cryptoxanthin; 4, Chl *a*; 5, β-Carotene; *, Unidentified compounds which absorb at both 442 and 650 nm. Unlabeled peaks in the A_{442} nm trace are unidentified carotenoids.

PSII they soon adjust to the new conditions and become more efficient in using the predominant wavelengths (2, 3, 40). Recent studies indicate that this is also the case for cyanobacteria (25, 28). The changes in relative numbers of PSI, PSII, and PBsomes which we have observed provides an explanation for this phenomenon. For cultures placed under RL, an increase in the numbers of PSII and their attendant RL-absorbing Chl (relative to PSI) without increasing numbers of the largely ineffective PBsomes (see Fig. 5) should allow a more equitable distribution of quanta to the two photosystems.

An earlier publication (13), using a different strain of *P. cruentum*, different culture conditions, a different assay for PSII (O_2 evolution), and light less specifically directed to PSII (orange rather than green light), reported a twofold increase (compared with our fivefold) in the ratio PSII/PSI for growth under PSI *versus* PSII light. The change in stoichiometry observed in this earlier work was due primarily to a change in cell content of PSI rather than, as we have found (Table IV), of PSII. The authors concluded, in addition, that the ratio PSII/PBsome was constant (though their data indicates PSII/PBsome is equal to about 2.5 in orange light and 3.0 in RL; 13, 30). In contrast, our results indicate a large variation in the ratio of PSII to PBsome. We found that RL-grown cultures contain 2.6 times more PSII per PBsome than cultures grown under GL (4.2 *versus* 1.6; Table IV).

Data from a study by Ley and Butler (21) on acclimation of *P. cruentum* to light of different spectral qualities is consistent with our results. In their work, an eightfold increase in the fraction of Chl attributed to PSII was accompanied by only a 1.6-fold increase in PE per Chl (see Fig. 3 of ref. 21). Since our data (Fig. 4; Table III) indicate constant antenna sizes for PSI, PSII, and PBsomes, the results of Ley and Butler can be interpreted as evidence of a fivefold increase in the ratio PSII/PBsome.

Several species of red algae, including *P. cruentum*, have been reported to contain about three to four PSII per PBsome (6, 18, 20, 30, 36) while other red algae and the cyanobacteria apparently contain only one or two PSII per PBsome, depending on the particular species (18, 22, 24, 30). Heretofore, the ratio of PSII centers to PBsomes has been regarded as invariant irrespective of the wavelength composition of the growth light or of other environmental parameters (11, 13, 24, 25, 30). We are aware of only one other report of an alteration in the stoichiometry of PSII and PBsomes. In mutant strain AN112 of *Synechococcus* 6301, a reduction of PBsome size to less than one-half of that in the wild-type was accompanied by a comparable reduction in the number of PSII per PBsome (from 1.9 down to 1.0; 22).

Antenna Sizes of PSI, PSII, and PBsomes

Our estimate of the Chl antenna size for PSI in *P. cruentum* (140 Chl) is similar to or just slightly higher than estimates made previously for cyanobacterial PSI (range of 120–140

Table IV. *Phycobilisome and Reaction Center Content and Stoichiometry*

Component or Ratio	Growth Light		
	Green	Red	White
		mol/10¹⁹ cells	
PSI (P_{700})	114 ± 8	75 ± 15	107 ± 16
PSII (Q_A)	29 ± 5	91 ± 22	57 ± 4
PBsome	18 ± 1	21 ± 3	20 ± 2
		mol/mol	
Q_A/P_{700}	0.26 ± 0.05	1.21 ± 0.10	0.54 ± 0.06
Q_A/PBsome	1.6 ± 0.3	4.2 ± 0.6	2.9 ± 0.1
P_{700}/PBsome	6.4 ± 0.2	3.5 ± 0.3	5.4 ± 0.4
(Q_A + P_{700})/PBsome	8.1 ± 0.2	7.7 ± 0.8	8.3 ± 0.3
Chl/P_{700}	158 ± 5	227 ± 8	181 ± 8
Chl/Q_A	628 ± 93	188 ± 22	339 ± 33
Chl/PBsome	1020 ± 11	785 ± 46	982 ± 57

Data are mean ± SD of three to five independent experiments.

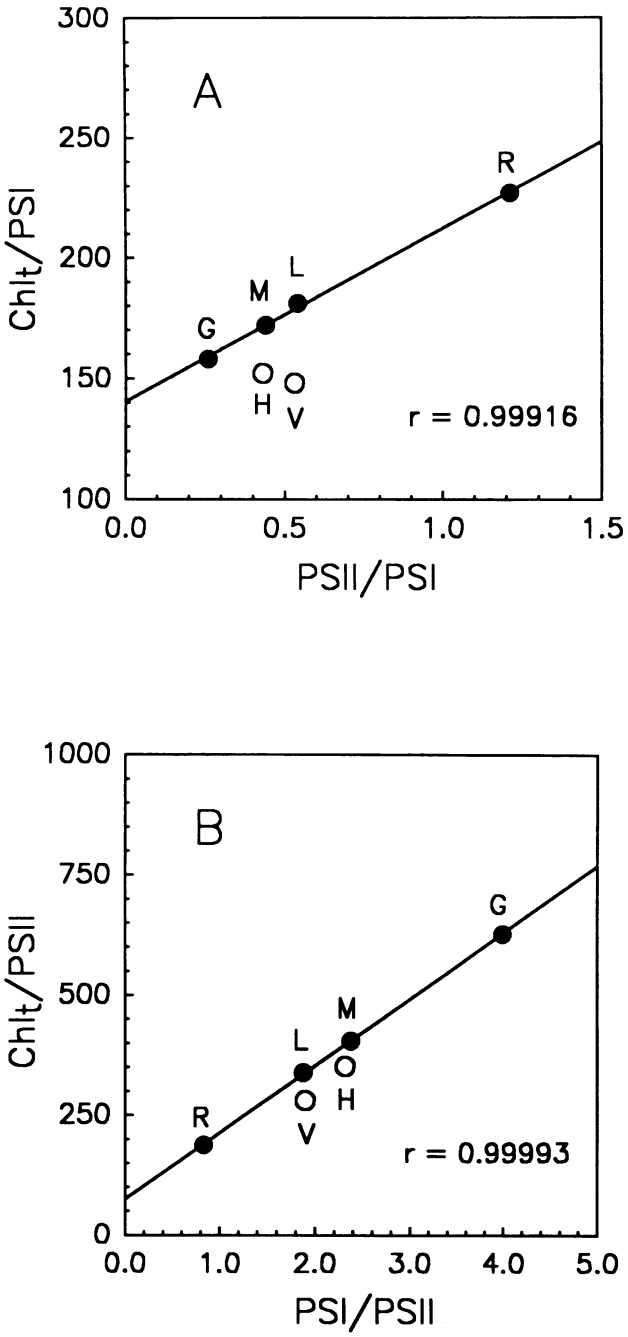


Figure 4. Plots of total Chl (Chl_t) per PSI or PSII as a function of photosystem stoichiometry in cultures of *P. cruentum* grown under a variety of light environments. (A), Chl_t per PSI as a function of PSII/PSI ratio; (B), Chl_t per PSII as a function of PSI/PSII. The ordinate intercepts (140.4 in A and 74.7 in B) indicate the Chl antenna sizes of PSI and PSII in those cultures whose data points fall along the regression line. Data points are mean values from three or four independent experiments for cultures grown under green (G) and red (R) light of 15 $\mu E \cdot m^{-2} \cdot s^{-1}$ (this work) or under WL of 6 (L), 35 (M), 180 (H), and 280 (V) $\mu E \cdot m^{-2} \cdot s^{-1}$ (6).

Chl; 11, 22, 29). The Chl antenna size calculated for PSII (75 Chl), however, is appreciably larger than other estimates for this photosystem in cyanobacteria (range of 20–50 Chl; 22, 26, 29) and in *Cyanidium* (35 Chl; 10), a putative red alga. The earlier figures are 'functional' estimates, derived by first estimating the numbers of PSI and PSII centers and then determining the proportion of the total quanta absorbed by Chl which results in PSII activity compared to that which engenders PSI activity. What we calculate is simply the number of Chl which behave as if associated with each PSII. A recent study on *Synechocystis* PCC 6714 (11), using a method similar to that used here, reported a value (about 60) which is more in line with our estimate for PSII antenna size in *P. cruentum* (75 Chl). Are these higher estimates simply a consequence of the uncertainties and assumptions inherent in the different methods or do they reflect a real difference in the 'functional' antenna size compared to the number of Chl which depend on PSII for their existence?

PSII of cyanobacteria and red algae are widely regarded as homologous to PSII of higher plants and green algae in which the accessory light-harvesting Chl *a/b* proteins are lacking (10). We have detected polypeptides of the appropriate apparent molecular weight in *P. cruentum* which are immunologically related to Chl-binding proteins of higher plant PSII 'cores' (using antibodies to D1, D2, CP43, and CP47 of spinach; our unpublished data). Estimates of the Chl content of PSII cores from higher plants are likewise in the range of 35 to 50 Chl, whether based on functional antenna size (*e.g.* 16) or measured in isolated PSII core particles (*e.g.* 35). However, cyanobacteria contain (in addition to CP47, CP43, D1, and D2) Chl-binding proteins of uncertain function which have been found to copurify with PSII preparations (7, 32). We speculate that additional Chl-binding proteins in red algae and cyanobacteria may serve as a conduit or bridge allowing the flow of energy from PSII to PSI (or from PBsome to PSI). Quanta absorbed directly by the Chl associated with these Chl-binding proteins may be available only to PSI (and thus explain why estimates of PSII Chl antenna size using our method give substantially higher values than functional estimates), but the assembly of this light-harvesting component would be dependent on and stoichiometric with PSII.

Arrangement and Interaction of Phycobilisomes and Photosystems

In the photosynthetic membranes of red algae and cyanobacteria, the photosystems are not sequestered in grana (PSII) and stromal (PSI) regions as is typical in higher plants and green algae. Based on energy transfer characteristics *in vivo* (10, 20–25), freeze-fracture studies of putative PSII particles (19, 36, 38), and the isolation of functionally coupled PBsome-PSII preparations (5), it is believed that PSII and PBsomes are functionally and structurally associated. Thus, the distribution of PSII in the thylakoid membrane may be defined by the arrangement of the PBsomes. It is possible that a subpopulation of PSII in the membrane are not connected to PBsomes. There are mutants of cyanobacteria which apparently lack PBsomes but retain functional PSII (4, 38). But a report that half of the PSII in *Cyanidium caldarium* are not

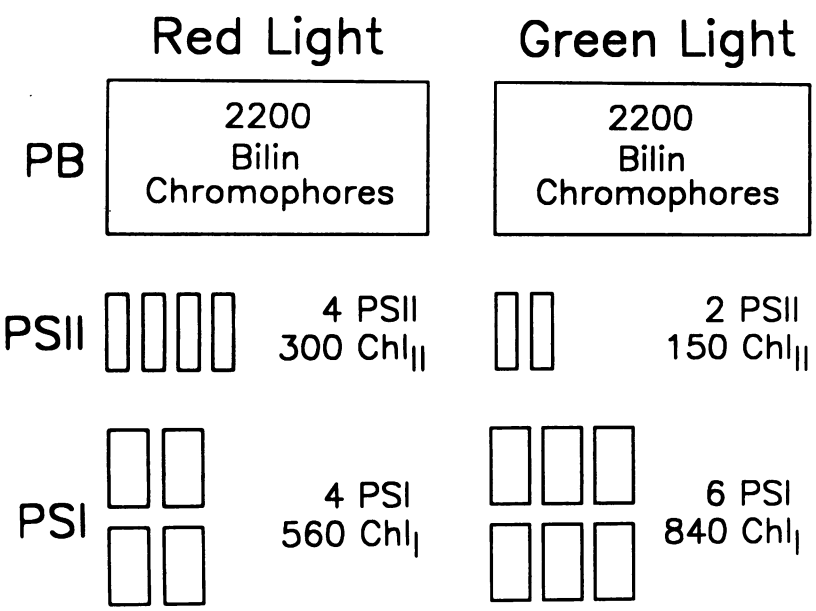


Figure 5. Schematic illustration of the relative numbers of PSI and PSII per phycobilisome (rounded to the nearest whole number from the data in Table IV) in cultures of *P. cruentum* grown under PSI light (RL) or PSII light (GL). Each is drawn to scale according to Chl content (75 Chl/PSII and 140 Chl/PSI) or phycobilin chromophore content. For calculation of phycobilin chromophore content it was assumed that each phycobilisome (PB) contains 58 PE, 12 PC, and 8 APC hexamers (see "Materials and Methods" and Table III), and that one-half of the PE had a gamma subunit (see reference 15 for phycobiliprotein chromophore content). Notice that the reduction in the number of PSII per phycobilisome under GL is accompanied by a complementary increase in the number of PSI.

functionally coupled to PBsomes (9) is probably a consequence of the protocol used. In that study (9), cells were cultured at 39°C but measurements were made at only 22°C. The work of Manodori and Melis (23) and Schreiber (34) indicate that half of the PSII in cyanobacteria may be reversibly uncoupled upon chilling of the cells. In *P. cruentum*, Ley (20) provides evidence that most, if not all, of the PSII reaction centers in WL-grown cells are functionally attached to PBsomes.

The range in stoichiometry we have observed for PSII and PBsomes (from 1.6–4.2 PSII per PBsome) leads us to hypothesize that up to four PSII may be bound to each PBsome and that a minimum of two are present with changes occurring as the loss or gain of a pair (Fig. 5). We note that Staehelin *et al.* (36) reported that the 100 nm EF particles (putative PSII centers) observed by freeze-fracture electron microscopy of thylakoid membranes in the red alga *Griffithsia monilis* tended to occur in groups of two or four. Also, PSII can be purified from *Synechococcus* sp. (7) in the form of a dimer. If correct, our hypothesis leads to the prediction of two distinct populations of PSII which may be differentiated by their phycobiliprotein antenna size. Experiments to test this prediction are contemplated.

The distribution of PSI in the thylakoid membrane and whether some or all of them are intimately associated with PBsomes are unknown. Likewise, we do not know if other membrane-bound macromolecular complexes such as the ATP synthase or the Cyt b_6f complex are confined to particular domains of the thylakoid membranes of red algae or cyanobacteria. That PSI receives energy initially absorbed by phycobiliproteins is evidenced by the high quantum yield of oxygen evolution under light absorbed primarily by PBsomes (2, 3, 28, 40), by action spectra of PSI (based on enhancement; 37), and by excitation spectra for fluorescence emission at wavelengths believed to arise from Chl of PSI (1, 21). Whether the energy flow is directly from PBsomes to PSI or occurs via PSII is still disputed (1), but PSI, or at least some fraction of them, must be in close physical proximity to the core of a PBsome or to a PSII center associated with the core in order to allow energy transfer. The constancy of the total number of photosystems (PSI + PSII) per PBsome (Table III; Fig. 5) may simply be fortuitous, but it is suggestive of a more strict association of PSI with the PBsome. The precise nature of the interactions between PBsomes and photosystems remains to be elucidated.

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