

Stoichiometry of Photosystem I, Photosystem II, and Phycobilisomes in the Red Alga *Porphyridium cruentum* as a Function of Growth Irradiance¹

Francis X. Cunningham, Jr., Ronald J. Dennenberg, Laszlo Mustardy², Paul A. Jursinic, and Elisabeth Gantt*

Department of Botany, University of Maryland, College Park, Maryland 20742 (F.X.C., L.M., E.G.); Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604 (R.J.D., P.A.J.)

ABSTRACT

Cells of the red alga *Porphyridium cruentum* (ATCC 50161) exposed to increasing growth irradiance exhibited up to a threefold reduction in photosystems I and II (PSI and PSII) and phycobilisomes but little change in the relative numbers of these components. Batch cultures of *P. cruentum* were grown under four photon flux densities of continuous white light; 6 (low light, LL), 35 (medium light, ML), 180 (high light, HL), and 280 (very high light, VHL) microeinsteins per square meter per second and sampled in the exponential phase of growth. Ratios of PSII to PSI ranged between 0.43 and 0.54. About three PSII centers per phycobilisome were found, regardless of growth irradiance. The phycoerythrin content of phycobilisomes decreased by about 25% for HL and VHL compared to LL and ML cultures. The unit sizes of PSI (chlorophyll/ P_{700}) and PSII (chlorophyll/ Q_A) decreased by about 20% with increase in photon flux density from 6 to 280 microeinsteins per square meter per second. A threefold reduction in cell content of chlorophyll at the higher photon flux densities was accompanied by a twofold reduction in β -carotene, and a drastic reduction in thylakoid membrane area. Cell content of zeaxanthin, the major carotenoid in *P. cruentum*, did not vary with growth irradiance, suggesting a role other than light-harvesting. HL cultures had a growth rate twice that of ML, eight times that of LL, and slightly greater than that of VHL cultures. Cell volume increased threefold from LL to VHL, but volume of the single chloroplast did not change. From this study it is evident that a relatively fixed stoichiometry of PSI, PSII, and phycobilisomes is maintained in the photosynthetic apparatus of this red alga over a wide range of growth irradiance.

An ability to adjust the composition of the photosynthetic apparatus to achieve a more efficient harvesting of light energy is expected to be of significant advantage to organisms which are subjected to long-term variations in the light environment (22). An extensive literature has accumulated on the physiological responses of marine algae and other photosynthetic organisms to changes in intensity and wavelength of the incident light (1, 22). A number of studies have examined

this phenomenon in the Rhodophyceae and, in particular, in the unicellular red alga *Porphyridium cruentum* (2, 3, 17, 20, 24–26, 38). These earlier studies have examined acclimation in *P. cruentum* primarily from the perspective of changes in function or activity, including such aspects as action spectra (38) effective absorption cross-sections (25) fluorescence yield spectra (26) and photosynthesis-irradiance curves (24). From previous work in our laboratory (24), it appears that growth under continuous light of a certain, fixed intensity results in physiological changes of advantage to *P. cruentum*. Cultures grown under low light intensities exhibit a very low compensation point which allows for a net carbon gain under severely limiting quantum flux. Conversely, those cultures grown under high PFD³ have a much greater photosynthetic capacity.

The present work was undertaken to obtain information on changes in the photosynthetic light-harvesting apparatus which accompany the previously observed changes in photosynthetic performance. Earlier studies on photoacclimation in *P. cruentum* (2, 3, 24, 26) measured PE/Chl ratios as a way to monitor changes in the light-harvesting apparatus. However, such observations are of limited value in understanding the underlying physical reorganization which occurs as a result of the acclimation process. Such an understanding requires detailed knowledge of changes in the numbers, sizes, and composition of the light-harvesting components for cultures grown under carefully defined and controlled conditions. Until now this information has been lacking. In this paper we present data on the total and relative numbers of PB and reaction centers, and on their antennae sizes and pigment composition as a function of growth irradiance (6–280 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in exponentially-growing batch cultures of *P. cruentum* (ATCC 50161). We show that growth irradiance levels do not cause any significant changes in the stoichiometric relationship of the photosystems and PB.

MATERIALS AND METHODS

Cell Growth

One liter batch cultures of *Porphyridium cruentum* (ATCC 50161) were grown in an artificial seawater medium (18) in

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² Permanent address: Institute of Plant Physiology, Biological Research Center, Hungarian Academy of Sciences, P.O. Box 521, Szeged, Hungary H-6701.

³ Abbreviations: PFD, photon flux density; APC, allophycocyanin; HL, high light of 180 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; LL, low light of 6 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; ML, medium light of 35 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; P_{700} , reaction center of PSI; PB, phycobilisome; PE, phycoerythrin; Q_A , primary quinone acceptor of PSII; PC, phycocyanin; VHL, very high light of 280 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

Fernbach culture flasks (Pyrex brand, 2500 mL capacity). Flasks were supplied with 5% CO₂ in air through a plug of sterile cotton at about 1 to 2 bubbles per s from a glass tube of 3 to 4 mm i.d., and shaken on a rotary platform shaker at 80 cycles/min at a constant temperature of 18°C.

Cultures were illuminated continuously from above with light provided by daylight fluorescent lamps (Sylvania F 48T12/D/VHO, 115 W). Light intensity was controlled by the number of lamps and the distance of the lamps from the culture flasks. PFD at the surface of the culture medium were measured with a cosine-corrected quantum sensor (Licor). Total depth of the culture medium was about 2 cm. Measurements were not corrected for the additional PFD contributed by reflection from the white surface of the shaker platform below the culture flasks.

Stock cultures of *P. cruentum* were grown at 35 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Cells were acclimated to other PFD over the course of four weeks and several generations with transfers to fresh medium, as necessary, in order to maintain cultures in the exponential phase of growth. For cultures grown at higher PFD, the light intensity was increased in stages to avoid the severe growth inhibition otherwise observed.

Cell number was determined with a hemacytometer. All cultures were harvested in the exponential phase of growth (at about 3×10^6 cells/ml) in order to minimize self-shading and other effects which depend on the phase of growth in batch culture.

Electron Microscopy

Cells were pelleted by low speed centrifugation and then fixed in 1% (v/v) glutaraldehyde in 0.1 M NaPO₄ buffer at pH 7.2. Samples were subsequently postfixed in 2% (w/v) OsO₄ in the same buffer, dehydrated, and embedded in Epon. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips 300 electron microscope. Cell and chloroplast cross-sectional areas were determined from central sections by weighing the appropriate portions of photographic prints. Volumes were calculated from this data with the assumption that cells and chloroplasts approximate a sphere.

Isolation of Thylakoid Membranes

Cells were harvested by centrifugation at 2,600g for 5 min (4,000 rpm in a Sorvall GSA rotor), washed in deionized water at 16,000g for 10 min (10,000 rpm), and the pellets were drained well and weighed. The cell pellets were resuspended in ice-cold 50 mM NaPO₄ buffer at pH 7.0 in the ratio 1 g pellet plus 2 mL buffer. All subsequent steps were carried out at 4°C or on ice.

Cells were passed through a French pressure cell (AMINCO) three times at 20,000 psi in order to ensure complete breakage of all cell types. Aliquots were frozen at -80°C for later use. The remainder of the pressate was centrifuged for 1 h at 178,000g (50,000 rpm in a Beckman 60 Ti rotor). The resulting pellets were resuspended in 10 mM NaPO₄ buffer (pH 7.0) using a glass pestle homogenizer, and sodium EDTA (from an 80-fold stock concentrate adjusted to pH 7.0 with NaOH) was added to give a final concentration of 2.5 mM. Ten mL of this solution were layered onto a sucrose step gradient

consisting of 6 mL of 1.6 M, 12 mL of 0.8 M, and 10 mL of 0.5 M sucrose, all in 10 mM NaPO₄ at pH 7.0. Gradients were centrifuged for 3 h at 96,000g (27,000 rpm in a Beckman SW 27 or SW 28 rotor). Thylakoid membranes were removed from the interface of the 0.8 and 1.6 M sucrose layers, diluted with an equal volume of 10 mM NaPO₄ buffer (pH 7.0), and centrifuged for 30 min at 178,000g (50,000 rpm in the 60 Ti rotor). Pellets were homogenized in the same buffer containing 0.5 M sucrose. Aliquots were stored at -80°C.

Pigment Determinations by Spectroscopy

Chl was determined in *N,N*-dimethylformamide (ACS grade from Sigma) using the extinction coefficient of Moran of 83.9 $\text{mm}^{-1} \cdot \text{cm}^{-1}$ at 664 nm for Chl *a* (30). The final concentration of *N,N*-dimethylformamide was never less than 95% (v/v) after addition of the aqueous sample.

PE was determined in 50 mM NaPO₄ buffer at pH 7.0 using the extinction coefficient of Gantt and Lipschultz of 5.26 $\text{ml} \cdot \text{mg}^{-1} \cdot \text{cm}^{-1}$ at 545 nm in 50 mM NaPO₄ at pH 7.0 (30) after a small correction (about 1–2%) for that portion of the *A*₅₄₅ due to Chl (*A*₅₄₅ - 0.2 × *A*₆₇₈). Cells broken in the French pressure cell (see "Isolation of Thylakoid Membranes") were diluted in buffer to about 20 to 60 μg PE per mL and allowed to sit at room temperature in the dark for 1 to 2 h. The samples were mixed well and then centrifuged at 129,000g (45,000 rpm in a Beckman Type 65 rotor) for 30 min at 20°C. The supernatant fluid was used to assay for PE. PB numbers were calculated from the spectroscopically determined levels of PE by assuming 60 PE hexamers (250,000 g/mol of hexamer) in each PB (9) for LL-grown cultures. It was assumed that PB in ML, HL, and VHL-grown cultures contain only 57, 44.4, and 47.4 PE hexamers, respectively, based on the deficiency of PE relative to APC in these cells compared with LL-grown cells (Table IV).

Pigment Determinations by HPLC

Carotenoids and Chl *a* were determined by HPLC on an Altex Ultrasphere ODS column (5 μm particle size and 4.6 mm by 15 cm column) with a 9 μL flow cell using a mobile phase gradient of increasing acetone in water (100:18 to 100:4 acetone to water, v/v) with the absorbance detector set at 442 nm. Outgassing in the flow cell was minimized by degassing of the mobile phases in a sonicating bath for 10 min just prior to use, and by maintaining a pressure of 50 psi with a backpressure regulator. Samples of thylakoids or broken cells ("French pressates") were diluted appropriately in deionized water and 100 volumes of acetone were then added to 17 volumes of the diluted sample. The final concentration of Chl was between 30 and 50 $\mu\text{g}/\text{mL}$. Extraction was allowed to proceed for about 20 min at room temperature in the dark, and the insolubles were removed by centrifugation at maximum speed for five min in a Beckman microfuge 12. The extract was filtered through a 0.45 μm nylon-66 membrane filter and injected (20 μL loop) onto the column with the mobile phase flowing at 0.8 mL/min. Authentic zeaxanthin (Roth), β -carotene and Chl *a* (both from Sigma) were further purified (to apparent homogeneity) by HPLC and used as standards to identify (by comparison of spectra and HPLC

retention time) and quantify (by integrated peak area compared to standard curves) the pigments in the crude sample extracts. Cell concentrations of the individual pigments were calculated with reference to the cell Chl content determined by spectroscopy.

Reaction Center Determinations

The concentrations of the PSI reaction center (P_{700}) and the primary quinone acceptor of PSII (Q_A) were determined in thylakoid membranes (at 20–25 $\mu\text{g Chl/mL}$) by the photochemically-induced absorbance changes at 701 nm and 325 nm, respectively, as previously described (8). An additional xenon flash lamp was necessary to saturate the absorbance change at 325 nm. The light energy delivered to the cuvette was $0.08 \text{ J} \cdot \text{cm}^{-2} \cdot \text{flash}^{-1}$.

Rocket Immunoelectrophoresis

The method of Plumley and Schmidt (34) was used with 1% (w/v) agarose gels (Bio-Rad DNA grade) 1.8 mm in thickness containing 4.5% (w/v) polyethylene glycol 3350 (Sigma). French pressates of whole cells (see "Isolation of Thylakoid Membranes") were heated at 100°C for 2 min in a solution containing 4% (w/v) SDS (Bio-Rad) and the gel buffer at 2 times the final concentration. After cooling, an equal volume of 25% (v/v) Triton X-100 (Sigma) was added to the sample which was subsequently microfuged for 5 min at maximum speed in a Beckman microfuge 12 in order to pellet insolubles. Duplicate samples of 8 μL each were loaded into wells approximately 2.3 mm in diameter and subjected to electrophoresis for 16 h at 150 V (5 V/cm). Gels were washed several times in 0.15 M NaCl, then in deionized water, and subsequently fixed and stained in 0.1% (w/v) Coomassie blue R-250 in aqueous 50% (v/v) MeOH and 10% (v/v) glacial acetic acid. Gels were destained in 20% (v/v) MeOH and 10% (v/v) acetic acid and then photographed using Polaroid type 55 P/N film. Samples were quantified by comparison of rocket area (determined by cutting out and weighing rockets from duplicate photographic enlargements made on Kodabrome II RC F5 paper) with standard curves constructed from serial dilutions of purified antigens and PB. Antisera to the phycobiliproteins (PE, PC, and APC) were obtained as previously described (14).

Purification of PB

PB were prepared as previously described (13), and were purified further by incubation with stirring overnight at room temperature in 1% (v/v) Triton X-100 (Sigma) in 0.5 M NaPO_4 buffer at pH 7.0. This was followed by a second gradient centrifugation step as before.

RESULTS

Cell Growth and Morphology

Cultures of *P. cruentum* were grown under continuous light at four PFD which encompass nearly the entire range under which this organism will grow and divide. Cultures growing under the lowest PFD of $6 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (LL) require nearly a

week to double in cell number (Table I). A sixfold increase in PFD to $35 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (ML) reduces the generation time to 36 h, or about one-fourth of that under LL. The generation time is further reduced to about 18 h under $180 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (HL), but an additional increase in PFD to $280 \text{ mE} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (VHL) is slightly inhibitory, with a generation time of about 22 h observed for these cultures.

Growth irradiance was found to have a significant effect on the total cell volume of *P. cruentum* (Fig. 1 and Table I). Increase in PFD was accompanied by an increase in cell volume over the range of PFD examined, with ML cells measured at about twice the volume and VHL cells at about three times the volume of cells grown under LL. At least part of the increased volume is due to a more extensive vacuolation and an increase in starch granules in cells grown under the higher PFD (see Fig. 1).

In contrast to the changes in cell volume, chloroplast volume did not vary appreciably with change in growth PFD under our culture conditions (Fig. 1 and Table I). Chloroplasts of ML cultures may be a little larger than those of cultures grown under the other PFD. The proportion of the cell volume occupied by the chloroplast in HL and VHL cells, about 30%, is thus considerably smaller than the 72% found for LL cells (Table I).

Chloroplasts of cells grown under the different PFD exhibit obvious differences in morphology (Fig. 1). The amount of thylakoid membrane within the chloroplast decreases progressively with increase in PFD. Chloroplasts of cells grown under LL are, except for the volume occupied by the pyrenoid, completely full of densely packed thylakoid membranes arranged in parallel. As irradiance increases, eventually only the very outermost thylakoid membranes are closely packed and parallel. Given the roughly equivalent chloroplast volumes, it is apparent that increase in growth irradiance results in a decrease in total thylakoid membrane area per cell.

Cell Pigment Content

Cells of *P. cruentum* contain a relatively simple mixture of Chl and carotenoids. Figure 2 is an HPLC elution profile of acetone-soluble pigments extracted from a ML-grown culture. Chl *a* is the predominant Chl species in this organism. A minor, quite polar peak which elutes near the front also absorbs at 650 nm and is probably chlorophyllide *a*. The carotenoid composition is comprised largely of zeaxanthin (β, β -carotene-3,3'-diol) and β -carotene (β, β -carotene) with a small amount of cryptoxanthin (β, β -caroten-3-ol), the biosynthetic intermediate of β -carotene and zeaxanthin. Two additional minor carotenoids are evident in the elution profile of Figure 2, and as many as five more are readily observed using higher sample loads or increased detector sensitivity. The amounts of the minor bands, although individually quantified, have been added together and listed as unidentified carotenoids in Tables II and III.

Exponentially growing cultures of *P. cruentum* grown under LL or ML exhibit little difference in cell content of Chl, total or individual carotenoids, or PE (Table II) despite the great differences in their respective generation times and cell sizes (Table I). Cultures grown under the much higher PFD of HL exhibit a dramatic reduction in cell content of Chl, β -carotene,

Table I. Properties of the *P. cruentum* Cultures

Property	Growth Irradiance ($\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)			
	6	35	180	280
Chl (pg/cell)	1.72 ± 0.18^a	1.82 ± 0.15	0.56 ± 0.06	0.59 ± 0.03
Generation time (h)	150	36	18	22
Cell volume (μm^3)	54 ± 14	102 ± 28	140 ± 32	156 ± 37
Chloroplast volume (μm^3)	38 ± 8	55 ± 12	37 ± 14	41 ± 12
Chloroplast volume as a % of cell volume	74 ± 8	56 ± 10	31 ± 15	26 ± 7
Thylakoid membrane area ($\mu\text{m}^2/\text{cell}$)	630 ± 190	580 ± 180	300 ± 150	250 ± 100

^a Chl values are mean \pm SD of at least three independent experiments. Volumes and membrane areas are mean \pm SD of at least 20 separate measurements.

cryptoxanthin, and PE. These cells, with a generation time about one-half that of ML cells, contain only about one-fourth of the PE, one-third of the Chl, and one-half of the β -carotene and cryptoxanthin present in LL or ML cells. In contrast, zeaxanthin, by far the major carotenoid species in *P. cruentum*, decreases only slightly, if at all. A further increase in light intensity to $280 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ results in no further decrease in the levels of the various pigments per cell, and zeaxanthin may increase. Like zeaxanthin, the total amount of unidentified carotenoid per cell remains approximately constant at all growth PFD.

The decrease in cell pigmentation with increase in PFD is not a continual, proportionate response. From our data it is clear that little change in cell content of PE, Chl, or carotenoid pigments occurs between 6 and 35 or between 180 and $280 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. A similar pattern was evident in the data of Jahn *et al.* (17), also for *P. cruentum* grown under continuous illumination. Of the large drop in phycobiliproteins which they observed concomitant with an increase in growth PFD from 10 to $150 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, 80% occurred between 25 and $50 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (17).

It is informative to examine the pigment data from the perspective of changes in relative amounts of the various components (Table III). Zeaxanthin increases more than four-fold from only 20% (mol/mol) to nearly 90% of the amount of Chl, and from about one-half to nearly three-fourths of the total carotenoid pigment present in the cell when PFD is increased from 6 to $280 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Under HL and VHL the unusual situation of more carotenoid than Chl is obtained. The carotenoid content of *P. cruentum* was previously determined by Stransky and Hager (36), with a composition (relative amounts of Chl, zeaxanthin, β -carotene and cryptoxanthin) similar to that found by us for HL-grown cells (Table III).

PB Composition

PB of *P. cruentum* contain three major colored phycobiliprotein species: PE, PC, and APC (13). We measured the relative amounts of these pigments in *P. cruentum* in two ways. First, we purified intact PB, free of contaminating Chl, from the different cultures and compared their absorption spectra after normalizing at 650 nm, the absorption peak of

APC (Fig. 3). No difference in the spectra of PB from LL- and HL-grown cultures is observed at 625 nm, the absorption maximum of PC, but a gross difference is apparent in the region 450 to 600 nm, where PE has its absorption maximum. The difference spectrum LL minus HL results in the spectrum of B-PE (13). Both HL and VHL PB exhibited only about 75% of the absorbance at 545 nm found in LL or ML PB of equal A_{650} . Fluorescence excitation and emission spectra at room temperature (not shown) indicated that the PE present in VHL and HL PB was at least as well coupled as that in LL or ML PB, based on the absence of appreciable room temperature fluorescence emission at 582 nm (from uncoupled PE) with excitation at 545 nm.

We also used an immunological method to assay relative amounts of PE, PC, and APC in whole cells of *P. cruentum*. Because this method measures the apoprotein directly, errors arising from chromophore bleaching or variability are eliminated. Samples of both HL and VHL cultures contain only about 75 to 80% of the PE found in samples of LL and ML cultures of equivalent APC content (Table IV). This agrees closely with the 25% reduction in A_{545} observed in the spectra of isolated PB normalized at 650 nm (Fig. 3). At all growth PFD examined, PC and APC maintained a fixed stoichiometry in cells of *P. cruentum* (Table IV). Because APC is a core component of the PB and PE is the most peripheral phycobiliprotein (14, 15), we conclude that HL- and VHL-grown cells and the PB which they contain are deficient in PE relative to ML- and LL-grown cells and PB.

Reaction Center and PB Content and Stoichiometry

Cell contents of PSI, PSII, and PB in cultures of *P. cruentum* grown under high PFD are greatly reduced compared to the levels found in cells grown under relatively low irradiance (Table V). Yet, despite the pronounced changes observed in relative pigment content (Table III), the relative numbers of these components differ very little (Table V). All cultures contain between 0.43 and 0.54 photoactive PSII centers (measured as Q_A by the absorbance change at 325 nm) per photoactive PSI center (measured by a photochemical P_{700} assay; a chemical assay (20) gave comparable results; data not shown). LL and VHL cells may contain slightly more PSII per PSI than ML and HL cells.

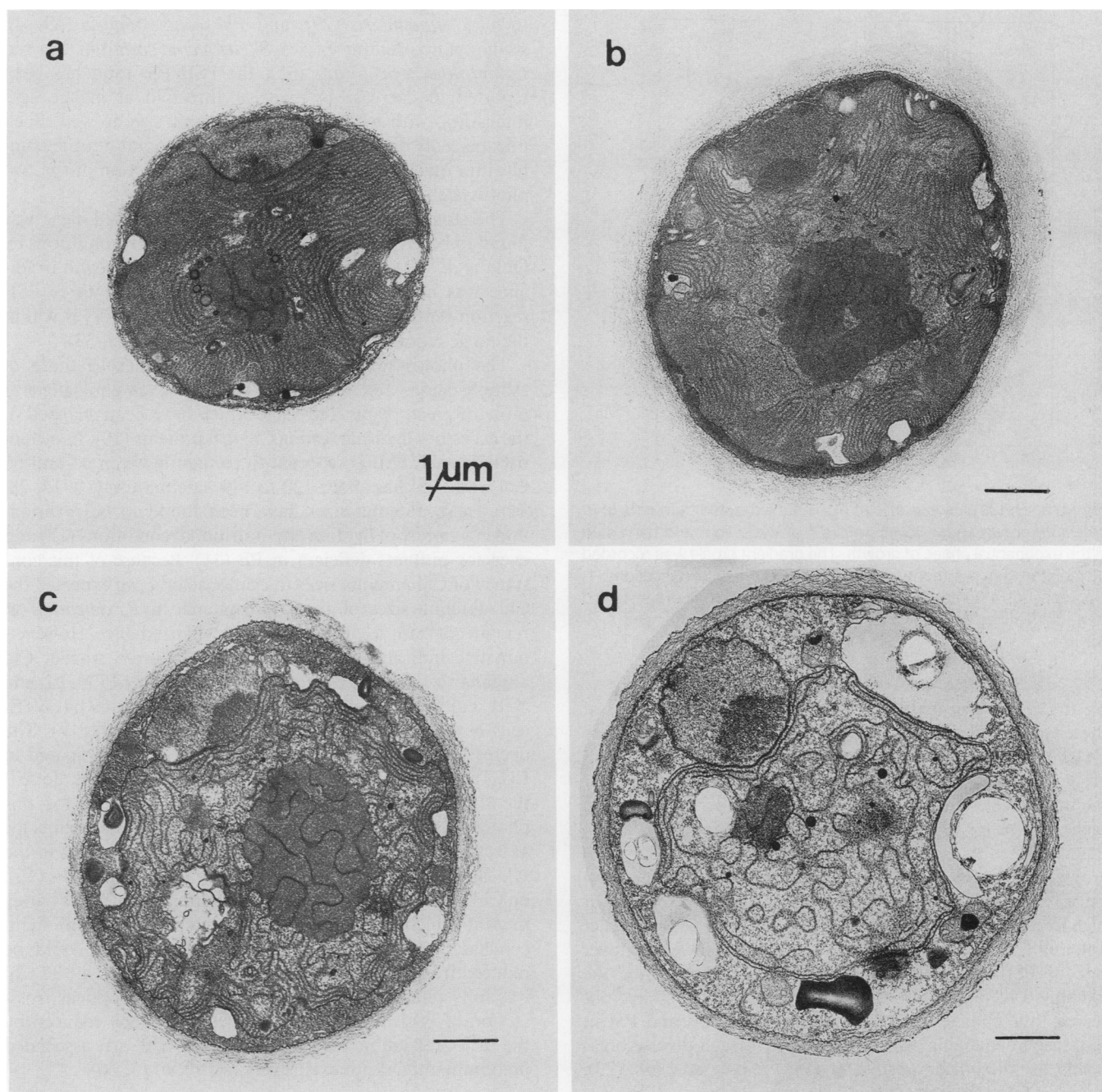


Figure 1. Electron micrographs of central sections of cells from exponentially growing cultures of *P. cruentum* grown under a range of white light intensities. All photographs are at the same magnification. Growth irradiance was: a, $6 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; b, $35 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; c, $180 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; and d, $280 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Notice especially that cell size increases with increase in PFD, but chloroplast size remains approximately constant and thylakoid density decreases.

PSII and its major light-harvesting antenna complex, the PB, occur in a relatively fixed ratio of about three to one (Table V). The higher value of 3.9 found for VHL cells may reflect damage to PSII centers since VHL is slightly inhibitory to growth (Table I).

Values for Chl per P_{700} and Chl per Q_A both decrease with increase in PFD (Table V). HL cells, with a ratio of Q_A per P_{700} equal to that of ML cells, have only about 88% of the

Chl per reaction center in ML cells. This value diminishes even further for VHL cells. We conclude that the Chl antenna of one or both of the photosystems is reduced in size under high growth irradiance.

DISCUSSION

Little data are available on the relative numbers of PSI, PSII, and PB in the red algae and none, as far as we know,

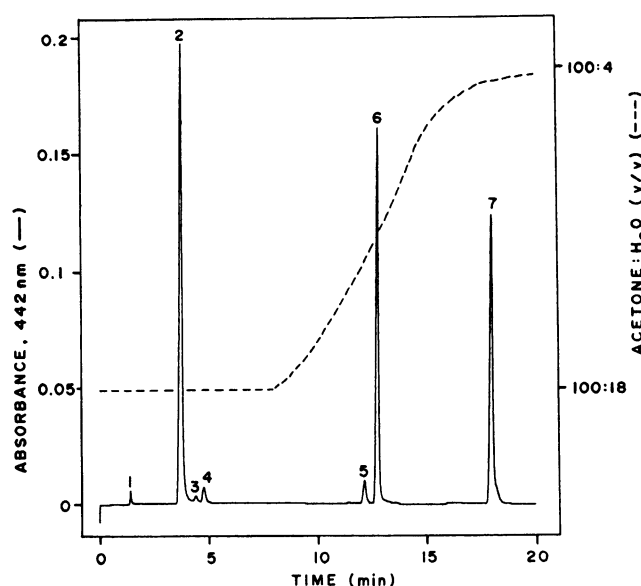


Figure 2. HPLC elution profile of pigments extracted from cells of *P. cruentum* grown under $35 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ of white light and harvested in the exponential phase of growth. The gradient profile was recorded by monitoring A_{330} without a sample injection. Peak identifications: 1, chlorophyllide a (tentatively); 2, zeaxanthin; 3, unidentified carotenoid A; 4, unidentified carotenoid B; 5, cryptoxanthin; 6, Chl a; and 7, β -carotene.

on the effect of growth irradiance on the stoichiometry of these complexes in Rhodophytes. The results from this study clearly show that the relative numbers of PSI, PSII, and PB are not altered in response to change in growth irradiance for the unicellular red alga *P. cruentum*. A number of studies on higher plants (6, 19), green algae (32) and cyanobacteria (20) have reported that the ratio of PSII to PSI becomes larger as growth irradiance increases. The unit size of PSII often diminishes as well. It has therefore been suggested that under high irradiance more PSII (relative to PSI), each with a smaller antenna size, are necessary to overcome the slower turnover rate of PSII relative to that of PSI (32) and reduce the likelihood of photoinhibition (6). However, others have observed little change in the stoichiometry of PSII and PSI in response to changing growth irradiance in such diverse organisms as *Dunaliella tertiolecta* (11), *Pisum sativum* (23),

Atriplex triangularis (29), and *Phaseolus vulgaris* (29). In studies of two diatom species, *Skeletonema costatum* (11) and *Cylindrotheca fusiformis* (35), the PSII/PSI ratio has been reported to decrease by two- or threefold at higher light intensities, with a decrease in PSII unit size as well. Such unexpectedly disparate responses serve to point out the complexities involved in the function and interaction of the two photosystems.

The stoichiometry of PSII to PB in several red algae was found to be invariant under a variety of growth conditions by Ohki *et al.* (33), although the effect of varying growth irradiance was not examined. Our finding of about three PSII reaction centers per PBS for *P. cruentum* (Table V) is within the range reported by others for red algae (21, 25, 33).

The photosystems of red algae are similar to those of cyanobacteria, and have been considered as equivalent to those of green plants and algae which have been stripped of the accessory light-harvesting Chl *a/b* proteins (10). In cyanobacteria each PSII is reported to contain between 35 and 60 Chl while PSI has about 120 to 140 antenna Chl (10, 12, 28, 31). These antenna sizes have been found to be invariant under a variety of lighting and nutritional conditions (12) and even for mutants deficient in PB (31). Because of this constancy of Chl antenna sizes in cyanobacteria, we expected the Chl antenna sizes of the photosystems in *P. cruentum* to remain constant regardless of the growth irradiance. However, our data indicate that PSI and/or PSII have a smaller Chl antenna size in cultures grown under the higher PFD. LL and VHL cultures exhibit comparable PSII/PSI ratios but VHL cells have 20% less Chl per reaction center (Table V). The decrease in Chl (60 fewer Chl per Q_A for VHL compared to LL; Table V) is too great to be accounted for by PSII alone if the Chl antenna size of 35 to 60 Chl expected for this photosystem (10, 12, 28, 31) is correct. PSI thus accounts for at least some, and perhaps all, of the antenna Chl loss in the HL- and VHL-grown cells. A study by Glick and Melis (16), on Chl antenna sizes of the photosystems in a Chl *b*-less barley mutant which had been greened under intermittent light, concluded that the "core" antenna Chl of PSI could be reduced from 130 to 95 but the PSII "core" of about 37 Chl was fully retained. It is worth noting that the optical cross-section of PSII in HL and VHL cells is already reduced by the 25% reduction of PE because the PB are associated predominantly, if not exclusively, with PSII (5, 26).

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

Pigment	Growth Irradiance ($\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)			
	6	35	180	280
	<i>mol/10¹⁷ cells</i>			
Chl	192 ± 21 ^a	203 ± 17	62 ± 5	66 ± 4
Zeaxanthin	42 ± 7	52 ± 2	44 ± 4	57 ± 13
β -Carotene	32 ± 4	34 ± 2	17 ± 1	15 ± 3
Cryptoxanthin	3.0 ± 0.5	4.1 ± 1.2	1.9 ± 0.2	1.8 ± 0.6
Unidentified carotenoids	2.7 ± 0.3	3.5 ± 0.6	2.2 ± 0.2	3.4 ± 1.2
Total carotenoids	80 ± 11	93 ± 3	64 ± 6	76 ± 18
PE	11.8 ± 1.0	10.4 ± 1.3	2.7 ± 0.5	2.9 ± 0.4

^a All values are mean ± SD of three to five independent experiments.

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

Pigment	Growth Irradiance ($\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)			
	6	35	180	280
	<i>mol/100 mol Chl</i>			
Zeaxanthin	22 \pm 2 ^a	25 \pm 2	70 \pm 2	87 \pm 22
β -Carotene	17 \pm 1	17 \pm 1	27 \pm 1	22 \pm 5
Cryptoxanthin	1.5 \pm 0.1	2.0 \pm 0.7	3.0 \pm 0.1	2.8 \pm 0.8
Unidentified carotenoids	1.4 \pm 0.3	1.7 \pm 0.3	3.5 \pm 0.1	5.2 \pm 2.0
Total carotenoids	41 \pm 2	46 \pm 4	103 \pm 2	117 \pm 30
PE	6.1 \pm 0.4	5.0 \pm 0.2	4.3 \pm 0.4	4.4 \pm 0.5

^a All values are mean \pm SD of three to five independent experiments.

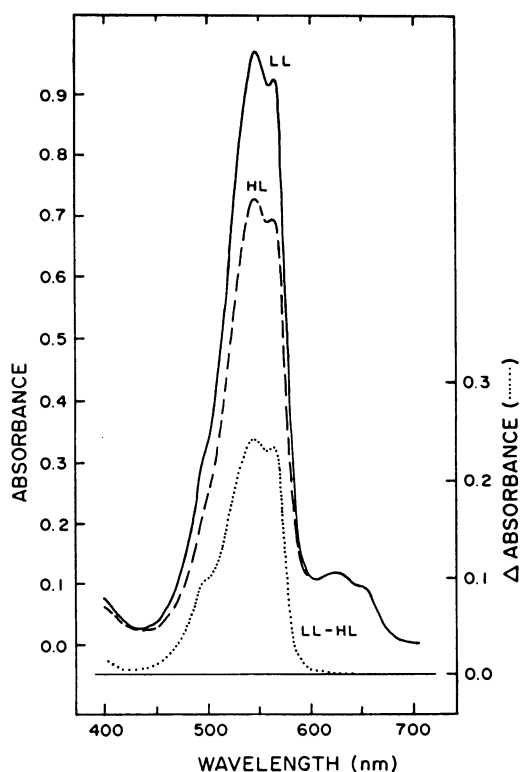


Figure 3. Absorption spectra of PB purified from cells of *P. cruentum* grown under 6 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (LL) or 180 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (HL) of continuous white light. Spectra were recorded in 0.5 M NaPO_4 buffer (pH 7.0) and normalized at 650 nm. The difference spectrum (LL-HL) indicates that PB from cells grown under 180 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ of light have less PE.

The effect of growth irradiance on individual carotenoids in photosynthetic organisms has received little attention. A few studies have been done in higher plants with the observation that β -carotene increases relative to xanthophylls when growth irradiance is increased (1, 27). Since β -carotene is associated largely with the reaction centers while much or most of the xanthophylls are found in the light-harvesting Chl *a/b* protein complexes of green plants, this data is understood as a reflection of increasing numbers of reaction centers relative to antennae complexes. In *P. cruentum* we observe the opposite response: a lower ratio of β -carotene to xantho-

phyll (largely zeaxanthin) as growth irradiance increases (Table II). *P. cruentum* has no Chl *b* and no accessory light-harvesting Chl *a/b* protein complexes. The location and function of zeaxanthin within the thylakoid membrane, if indeed that is its location, remain unknown. The lack of any correlation of the amount of zeaxanthin with Chl content, thylakoid area, or growth irradiance argues against an antenna function. It has recently been proposed that zeaxanthin is involved in the radiationless dissipation of excess light energy (7). Photoprotection may be its role in *P. cruentum* as well.

Many cyanobacteria exhibit pronounced changes in the relative amounts of the different phycobiliproteins within their PB in response to changes in environmental conditions, especially light wavelength and intensity but also as a result of deprivation or limitation of almost any nutrient (4). In contrast, red algal PB are generally considered to maintain a fixed size and composition under nutrient sufficient conditions. Brody and Emerson (2), for instance, reported that the PE to PC ratio remains constant in *P. cruentum* grown under a broad range of PFD. Exceptions to this generalization have been reported, however. In whole cells of the macrophytic red alga *Griffithsia pacifica*, Waaland *et al.* (37) reported a decrease in PE of 27% relative to PC and APC when growth irradiance was increased from 1 to 170 $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. In phycobilisomes of *Callithamnion roseum*, Yu *et al.* (39) found that the chromophore composition of PE was variable and changed in response to a change in irradiance. The amount of the apoprotein itself was not believed to change. Our results (Table IV and Fig. 3) indicate that the PB of *P. cruentum* are variable in size and composition, with a specific loss of the peripheral B-PE at higher PFD. The use of two complementary approaches has allowed us to show that the loss of B-PE chromophores in isolated BP is accompanied by the loss of the apoprotein itself in both the PB and whole cells.

The differences in photosynthetic performance which result from growth of *P. cruentum* under continuous low or high intensity white light (low compensation point and high photosynthetic capacity, respectively), are likely to involve more than simply a change in the amount of light-harvesting components. The amounts or activities of enzymes involved in other processes integral to photosynthesis; including photophosphorylation, reduction of NADP, and reduction of CO_2 ; may be affected as well. These will be considered in a forthcoming communication.

Table IV. Relative Amounts of Phycobiliproteins in *P. cruentum*

Phycobiliprotein ratio	Growth Irradiance ($\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)			
	6	35	180	280
	* % of mean for cells grown at $6\ \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$			
PE/APC (mol/mol)	100 \pm 4 ^a	95 \pm 2	74 \pm 1	79 \pm 3
PC/APC (mol/mol)	100 \pm 1	100 \pm 5	103 \pm 2	105 \pm 6

^a All values are mean \pm SD of three independent experiments. Phycobiliproteins were quantified by rocket immunoelectrophoresis.

Table V. PB and Reaction Center Content and Stoichiometry in *P. cruentum*

	Growth Irradiance ($\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)			
	6	35	180	280
	mol/10 ¹⁹ cells			
PSI (P ₇₀₀)	107 \pm 16 ^a	118 \pm 7	41 \pm 3	44 \pm 2
PSII (Q _A)	57 \pm 4	52 \pm 12	18 \pm 1	23 \pm 1
PB	20 \pm 2	19 \pm 2	6.0 \pm 1.1	5.8 \pm 0.7
	mol/mol			
Q _A /P ₇₀₀	0.54 \pm 0.06	0.45 \pm 0.11	0.43 \pm 0.02	0.53 \pm 0.01
Q _A /PB	2.9 \pm 0.1	2.8 \pm 0.4	3.0 \pm 0.7	3.9 \pm 0.4
Chl/P ₇₀₀	181 \pm 8	172 \pm 13	152 \pm 7	148 \pm 1
Chl/Q _A	339 \pm 33	405 \pm 67	352 \pm 33	281 \pm 2
Chl/PB	980 \pm 60	1130 \pm 50	1050 \pm 90	1110 \pm 110

^a All values are mean \pm SD of three to five independent experiments.

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