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Photoacclimation in the Red Alga *Porphyridium cruentum*[1]

Changes in Photosynthetic Enzymes, Electron Carriers, and Light-Saturated Rate of Photosynthesis as a Function of Irradiance and Spectral Quality

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ABSTRACT

Acclimation of the photosynthetic apparatus to changes in the light environment was studied in the unicellular red alga *Porphyridium cruentum* (American Type Culture Collection No. 50161). Absolute or relative amounts of four photosynthetic enzymes and electron carriers were measured, and the data were compared with earlier observations on light-harvesting components (F.X. Cunningham, Jr., R.J. Dennenberg, L. Mustárdy, P.A. Jursinic, E. Gantt [1989] Plant Physiol 91: 1179–1187; F.X. Cunningham, Jr., R.J. Dennenberg, P.A. Jursinic, E. Gantt [1990] Plant Physiol 93: 888–895) and with measurements of photosynthetic capacity. P_{max}, the light-saturated rate of photosynthesis on a chlorophyll (Chl) basis, increased more than 4-fold with increase in growth irradiance from 6 to 280 μeinsteins$\cdot m^{-2}\cdot s^{-1}$. Amounts of ferredoxin-NADP$^+$ reductase, ribulose-1,5-bisphosphate carboxylase, and cytochrome *f* increased in parallel with P_{max}, whereas numbers of the light-harvesting complexes (photosystem [PS] I, PSII, and phycobilisomes) changed little, and ATP synthase increased 7-fold relative to Chl. The calculated minimal turnover time for PSII under the highest irradiance, 5 ms, was thus about 4-fold faster than that calculated for cultures grown under the lowest irradiance (19 ms). A change in the spectral composition of the growth light (irradiance kept constant at 15 μeinsteins$\cdot m^{-2}\cdot s^{-1}$) from green (absorbed predominantly by the phycobilisome antenna of PSII) to red (absorbed primarily by the Chl antenna of PSI) had little effect on the amounts of ribulose-1,5-bisphosphate carboxylase, ATP synthase, and phycobilisomes on a Chl, protein, or thylakoid area basis. However, the number of PSI centers declined by 40%, cytochrome *f* increased by 40%, and both PSII and ferredoxin-NADP$^+$ reductase increased approximately 3-fold on a thylakoid area basis. The substantial increase in ferredoxin-NADP$^+$ reductase under PSI light is inconsistent with a PSI-mediated reduction of NADP as the sole function of this enzyme. Our results demonstrate a high degree of plasticity in content and composition of thylakoid membranes of *P. cruentum*.

The flux of photosynthetically active radiation in natural ecosystems varies both on a regular basis (e.g. diurnal and seasonal cycles) and unpredictably due to atmospheric and other, more localized, shading phenomena. The spectral "quality" of the available light also may vary, often as a result of shading by other plants and, in the case of aquatic plants, because of selective absorption by various materials suspended or dissolved in the water column (22).

Efficient oxygenic photosynthesis is thought to require the concerted action of two photosystems. In most plants these two photosystems have separate and, to varying degrees, spectrally distinct antenna systems. An alteration in the spectral distribution of the irradiance can, therefore, affect the balance of quantum capture by the two photosystems. An imbalance may result in a reduction in the quantum efficiency of photosynthesis (12, 18, 31).

In a variety of ways, oxygenic plants are able to adjust to changes in the light environment. In the short term, an excess or an imbalance in quantum capture by antennae of the two photosystems can be alleviated or corrected by such mechanisms as redistribution of accessory antenna complexes (4), adjustment in the degree of "spillover" from PSII to PSI (7), modulation of stromal enzyme activities (20), chlororespiration (9), and dissipation of excess energy (15, 17). In the longer term, many plants can "acclimate" to a newly prevailing light climate and grow more efficiently under those conditions by changing the content and/or composition of the photosynthetic apparatus (1, 2, 11, 12, 16, 18, 24, 26, 29, 36, 37).

Our interest is in understanding the structure, function, and plasticity of photosynthetic membranes in eukaryotic organisms that contain PBsomes[2]: the red algae. Our experimental subject is the unicellular red alga *Porphyridium cruentum* (ATCC No. 50161), which can grow well under PPFD much lower than that required by most green plants. Relatively little is known about the acclimation process in red algae, and the photosynthetic apparatus of these organisms differs in some important respects from those of the higher

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[2] Abbreviations: PBsome, phycobilisome; FNR, Fd-NADP$^+$ reductase; GL, green light of 15 $\mu E\cdot m^{-2}\cdot s^{-1}$; HL, high light of 180 $\mu E\cdot m^{-2}\cdot s^{-1}$; LL, low light of 6 $\mu E\cdot m^{-2}\cdot s^{-1}$; ML, medium light of 35 $\mu E\cdot m^{-2}\cdot s^{-1}$; P_{700}, reaction center of PSI; P_{max}, light-saturated rate of photosynthesis on a Chl basis; Q_A, primary quinone acceptor of PSII; RL, red light of 15 $\mu E\cdot m^{-2}\cdot s^{-1}$; WL, white light; VHL, very high light of 280 $\mu E\cdot m^{-2}\cdot s^{-1}$; ATCC, American Type Culture Collection.

plants and green algae that have been the subjects of most acclimation studies. The two photosystems of red algae (and of cyanobacteria) are much like the "cores" of those in green plants and algae (8). However, Chl *b*, accessory light-harvesting Chl *a/b* complexes, and lateral differentiation of the thylakoid membranes into granal and stromal lamellae are lacking in red algae and cyanobacteria. An extrinsic membrane-associated biliprotein complex, the PBsome, serves as the major accessory light-harvesting antenna system. The green and orange light-absorbing PBsomes of *P. cruentum* are associated with and transfer absorbed light energy to PSII (10). Most of the blue and red light-absorbing Chl is associated with PSI (13, 27). As a consequence of their nearly complementary absorption spectra, severe imbalances in quantum capture by the antenna of PSI and PSII are easily introduced. Under GL, for instance, as much as 97% of the quanta captured by the light-harvesting antenna of *P. cruentum* may be absorbed by the PBsome antenna of PSII (27).

In the work described in this paper, cultures of *P. cruentum* were allowed to acclimate to moderate and extremes of irradiance and spectral composition. We exploited the acclimation process, using it as a tool to manipulate the content and composition of the thylakoid membrane and thereby gain insight into how such changes affect photosynthetic performance. In previous work, we quantified levels of the three major light-harvesting complexes: PSI, PSII, and PBsomes (13, 14). Here, we examine the influence of the growth light on the amounts of ATPase, Cyt *f*, FNR, and Rubisco and on P_{max}. With these data we address such questions as: What are the constraints on or limitations to acclimation in *P. cruentum*? How plastic is the composition of the photosynthetic membrane in this red alga compared to the membranes of higher plants, green algae, and cyanobacteria examined in other studies (11, 16, 18, 26, 29, 31, 36, 37)? How does photosynthetic performance in *P. cruentum* vary with irradiance and spectral quality, and what compositional alterations occur and can account for the changes in performance? Are there any fixed stoichiometric relationships among the various enzymes, electron carriers, and light-harvesting components that might imply higher levels of organization?

MATERIALS AND METHODS

Cell Culture

Samples were obtained from exponentially growing batch cultures of *Porphyridium cruentum* (ATCC No. 50161) that had been maintained in the logarithmic growth phase and acclimated to the chosen light conditions for 4 weeks. Continuous illumination was provided by daylight fluorescent tubes (Sylvania), with RL or GL obtained by using the appropriate filters (13). Cultures were grown in an artificial seawater medium at 18°C, bubbled with 5% CO_2 in air, and shaken at 80 cycles/min. Additional details have been published (14).

Sample Preparation

Sample preparation was described in an earlier paper (14). Briefly, cells were harvested by centrifugation, washed quickly with distilled water, and resuspended in 50 mM sodium phosphate buffer at pH 7.0. Cell number was determined with a hemacytometer, and Chl was quantified by spectroscopy after extraction with *N,N*-dimethylformamide (μg Chl *a* per mL = 11.92 × $A_{664.5}$) (30). The final concentration of *N,N*-dimethylformamide was 95% (v/v). Cells were broken by three passes through a French pressure cell at 18,000 p.s.i., and aliquots of this cell pressate were frozen at −80°C. The remainder of the pressate was used for isolation of thylakoid membranes by sucrose gradient centrifugation. Purified thylakoids were stored in aliquots at −80°C in 50 mM sodium phosphate buffer containing 0.5 M sucrose.

Protein Assay

Total protein in cells of *P. cruentum* was estimated using a modified Lowry procedure (28) with BSA as the reference standard. Cells were broken in a French pressure cell as described above, protein and Chl were assayed in aliquots of the samples, and protein per cell was calculated from the previously determined Chl content of the cells.

Spectroscopic Assay of Cyt *f*

Cyt *f* was quantified by ascorbate-reduced minus ferricyanide-oxidized difference spectra (Fig. 1). Gradient-purified thylakoid membranes were dispersed in a solution containing 2% (v/v) Triton X-100 (Sigma) and 50 mM sodium phosphate

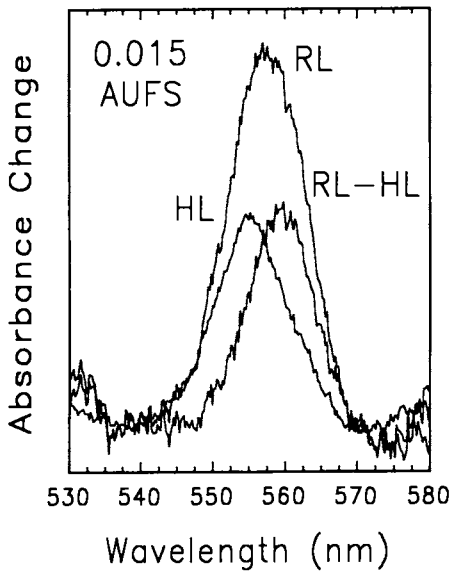

Figure 1. Ascorbate-reduced minus ferricyanide-oxidized difference spectra of thylakoid membranes of *P. cruentum* solubilized in 2% (v/v) Triton X-100 and 50 mM sodium phosphate buffer at pH 7.0. Each spectrum is the average of five repetitive scans. The difference spectrum labeled HL was obtained with thylakoids of cells grown under 180 $\mu E \cdot m^{-2} \cdot s^{-1}$ of continuous WL; that labeled RL was from thylakoids of cells grown under 15 $\mu E \cdot m^{-2} \cdot s^{-1}$ of continuous RL. The Chl concentrations of the samples (120 μM for RL and 54.8 μM for HL) were adjusted to give equal concentrations of Cyt *f*. Subtraction of the HL difference spectrum from the RL one then approximates a pure Cyt *b*-559_{HP} difference spectrum. AUFS, Absorbance units full scale.

at pH 7.0. After the solution was incubated for 5 min in darkness at room temperature, potassium ferricyanide (from a freshly made 100 mM stock in deionized water) was added to give a final concentration of 2 mM, and the solution was clarified by centrifugation for 5 min at maximum speed in an International Equipment Company model CL tabletop clinical centrifuge. The final solution typically contained 120 μM Chl and was distributed into four self-masked, semimicrocuvettes. The cuvettes were placed in the reference and three sample positions of the cell holder in a Gilford Response scanning spectrophotometer and allowed to sit in darkness for 3 min. Both bandwidth and scanning increment were set to 0.5 nm. Spectra (530–580 nm) were recorded both before and 4 min after the addition of a small amount of solid sodium ascorbate to the sample cuvettes. The changes in absorbance at 552 and 560 nm relative to a baseline drawn between isosbestic points at 538 and 570 nm in the difference spectra (referred to as the height or H) were determined. Concentrations of Cyt *f* were calculated using the equation of Bendall and Rolfe (5; [Cyt $f = (58.3 \times H_{552}) - (16.7 \times H_{560})$ nmol/mL]), to correct for the change in absorbance due to ascorbate-reducible Cyt *b*-559 (Fig. 1). If ascorbate-reducible Cyt *b*-559 is not of interest, then the use of hydroquinone (rather than ascorbate) would obviate the need for such a correction (5).

Determination of P_{max}

P_{max} was determined by measuring the rate of oxygen evolution at 18°C under saturating WL using a Hansatech model DW1 oxygen electrode apparatus. Details of the procedure were described by Lee and Vonshak (25).

Rocket Immunoelectrophoresis

Relative amounts of ATPase in cells of *P. cruentum* were quantified by rocket immunoelectrophoresis using a rabbit antiserum specific for the β-subunit of the spinach enzyme. This antiserum was generously provided by Dr. A. T. Jagendorf of Cornell University. The procedure we used is based on that of Plumley and Schmidt (35). Our minor modifications of their procedure are described in an earlier paper (14).

Immunoblotting

Immunoblotting was used to examine the specificity of the various antisera (32) and to quantify relative amounts of Rubisco and FNR in cells of *P. cruentum*. All antisera were from rabbits that had been inoculated with polypeptide antigens purified from spinach. An antiserum specific for the large subunit of Rubisco was provided by Dr. S.-D. Kung of the University of Maryland Center for Biotechnology and that for FNR was a gift from Dr. John Gray of Cambridge University.

Broken cells (see "Sample Preparation" above) were solubilized by heating at 100°C for 1.5 min in sample buffer (23) containing 2% (w/v) SDS. DTT (from a stock concentrate of 0.5 M in deionized water) was added to give a final concentration of 50 mM, and the polypeptides were separated by SDS-PAGE on 7 to 19% gradient gels using the buffer system of Laemmli (23). Polypeptides were electrophoretically blotted onto Immobilon-P membranes in a Hoefer TE 42 transphor cell at 40 V for 1 h and then 100 V for another hour. Gels were equilibrated and transferred in the buffers described by Peluso and Rosenberg (34). Immunostaining was performed using the Bio-Rad Immun-Blot alkaline phosphatase kit. Lanes were scanned using a Bio-Rad model 620 video densitometer in the reflectance mode, and data were analyzed using the 1-D Analyst Program provided by Bio-Rad. Relative amounts of Rubisco and FNR were estimated by comparison with standard curves plotted using data obtained by serial dilution of one of the samples. All gels included lanes loaded with this sample to serve as internal standards.

RESULTS

Irradiance Acclimation

Batch cultures of the unicellular red alga *P. cruentum* (ATCC No. 50161) were grown under continuous WL at four different PPFDs. These ranged from a low, limiting irradiance of 6 $\mu E \cdot m^{-2} \cdot s^{-1}$ (LL), which supported a growth rate only 12% of the maximum (Fig. 2), to a more moderate PPFD of 35 $\mu E \cdot m^{-2} \cdot s^{-1}$ (ML; 50% of maximum growth rate), a relatively high irradiance of 180 $\mu E \cdot m^{-2} \cdot s^{-1}$ (HL; maximum growth rate), and a very high, slightly inhibitory irradiance of 280 $\mu E \cdot m^{-2} \cdot s^{-1}$ (VHL). All cultures were allowed to acclimate to the chosen irradiance for 4 weeks and were continuously maintained in the exponential growth phase by frequent dilution in fresh growth medium.

In an earlier study (14), we reported that numbers of the three major light-harvesting complexes (PSI, PSII, and PBsomes), amounts of their component pigments (Chl and phycobilins), and thylakoid membrane area were all reduced substantially in cells of *P. cruentum* grown under saturating light (HL or VHL) compared with those grown under limiting light (LL or ML). Cell and chloroplast volumes were also affected by the growth irradiance. In Figures 2 and 3 we compare these earlier results with new data, obtained using aliquots of the same samples, on the amount of protein and on the levels of four photosynthetic enzymes and electron carriers.

To facilitate comparison, the data in Figure 2 (bottom) and in other figures were normalized to a value of 100 for cultures grown under the lowest irradiance. In addition, a logarithmic scale was used for the ordinate axis of these figures to ensure that the normalization procedure did not introduce a visual bias. Thus, for instance, any 2-fold change in the ordinate, whether a doubling (e.g. from 100 to 200) or a halving (e.g. from 100 to 50) will be of the same amplitude.

In Figure 3, the relative amounts of the various enzymes, light-harvesting complexes, and electron carriers are expressed on the basis of Chl, cell number, and protein. On a Chl basis, components of the light-harvesting system (PSI, PSII, and PBsomes) are relatively constant regardless of the irradiance. In contrast, amounts of ATPase, Rubisco, Cyt *f*, and FNR increase progressively with irradiance until light becomes saturating for growth (HL; Fig. 2, top). Additional input of quanta (VHL) does not result in an additional in-

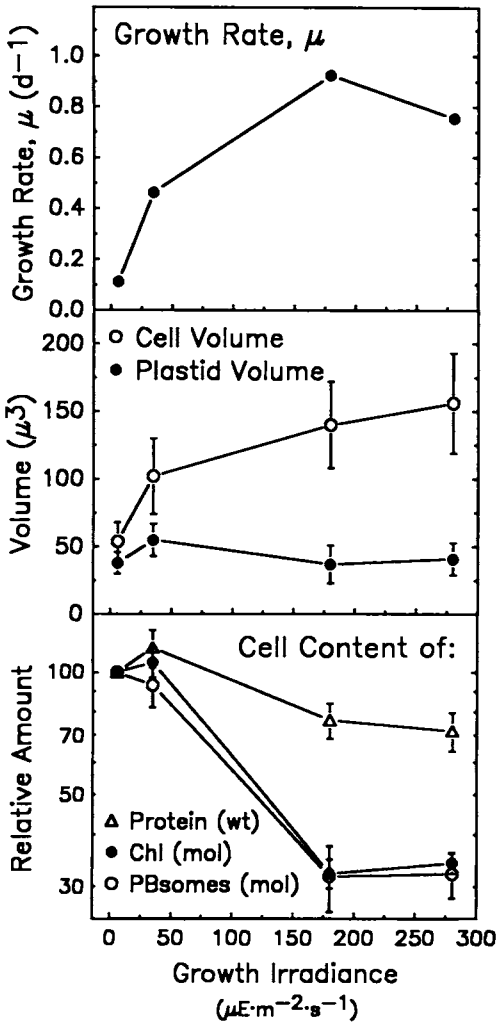

Figure 2. Influence of irradiance (PPFD) on growth rate (top), cell and chloroplast volumes (middle), and cell content of protein, Chl, and PBsomes (bottom). The growth rate is expressed as μ (ln 2/ generation time in days). Irradiance was continuous WL from daylight fluorescent tubes. Data for protein, Chl, and PBsomes are mean ± SD of at least three independent experiments. These data were normalized to a value of 100 for cells grown under 6 $\mu E \cdot m^{-2} \cdot s^{-1}$. A normalized value of 100 is equivalent to 56 μg of protein, 2.0 pmol of PBsomes, and 1.9 nmol of Chl per 10^6 cells.

crease in levels of these enzymes relative to Chl. The amount of enzyme per Chl is about 3-fold greater for Cyt *f* and FNR, 4-fold greater for Rubisco, and nearly 7-fold higher for ATPase in cultures grown under HL or VHL compared with those grown under LL. In sum, there is a substantial increase in photosynthetic enzymes and electron carriers, relative to antenna pigments and complexes, in cells grown under high irradiance compared with the levels in cells grown under low irradiance. But which are the variable components: the antenna pigments and complexes or the photosynthetic enzymes and electron carriers? The answer depends on the frame of reference.

On a cell basis (Fig. 3), the amounts of Rubisco, ATPase, Cyt *f*, and FNR are at a minimum under the lowest irradiance, and each of these, except ATPase, are at a maximum in cells grown under ML. Light-harvesting complexes (PSI, PSII, and PBsomes), in contrast, are at a minimum under saturating irradiance (HL and VHL) and a maximum under light-limited conditions (LL and ML). It appears that both antenna components and enzymes are variable, but interpretation of the results is complicated by a larger plastid volume in ML cells (45% larger than in LL, HL, and VHL cells; Fig. 2, middle).

We expressed the data on the basis of total protein as a way of avoiding the complications of a variable cell and plastid size and to examine the manner in which protein resources available to the cell are apportioned to the various components of the photosynthetic apparatus. The resulting plots are like those obtained with the data expressed on the basis of cell number, except that data points for ML cultures are lower. Amounts of Rubisco, FNR, and Cyt *f* per protein in ML cells are about equal to those under HL and VHL. From this perspective, one may view acclimation of *P. cruentum* to relatively high irradiance as involving primarily a reduction in light-harvesting capacity and thylakoid membrane area, with enzymes of photosynthetic electron transport and carbon fixation maintained at a constant proportion of the total protein in ML, HL, and VHL. ATPase is an interesting exception and increases in proportion to the growth rate (Fig. 4).

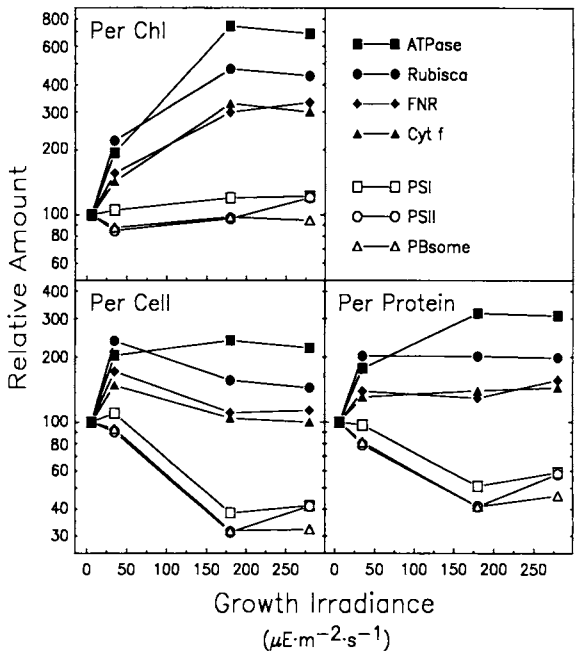

Figure 3. Photosynthetic enzymes and light-harvesting components of *P. cruentum* as a function of irradiance. Data are presented in relative units normalized to a value of 100 at the lowest irradiance (LL). A normalized value of 100 is equivalent to 5.5 PSI (P_{700}), 3.0 PSII (Q_A), 1.5 Cyt *f*, and 1.0 PBsome per 1000 Chl (mol/mol). Data points are means of at least three independent experiments. Error bars are omitted for clarity; SD values were ±5 to 10% or less. Quantitation was by spectroscopy (Cyt *f*, PSI, PSII), rocket immunoelectrophoresis (ATP synthase, PBsomes), or densitometry of immunoblots (Rubisco, FNR).

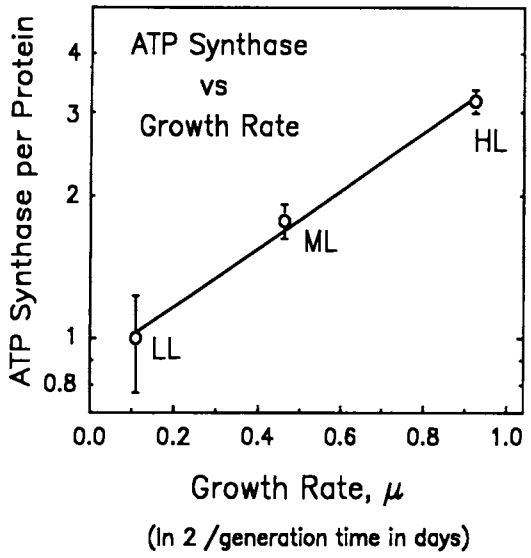

Figure 4. ATPase per protein versus the growth rate. Data were normalized to a value of 1 for LL-grown cultures.

Calculations using earlier estimates of thylakoid area per cell (14) and the data in Figure 3 provide some information concerning how the acclimatory changes in cell content of photosynthetic enzymes and light-harvesting components are manifested at the level of the thylakoid membrane. We estimate that concentrations of FNR, Cyt *f*, and ATPase in the thylakoid membrane were increased by nearly 3-fold (Cyt *f* and FNR) or 6-fold (ATPase) as irradiance was increased from LL to HL or VHL. The amount of Rubisco relative to thylakoid area increased almost 4-fold. The calculated concentrations of PSI, PSII, and PBsomes, on the other hand, were reduced by a relatively modest amount (20–35%).

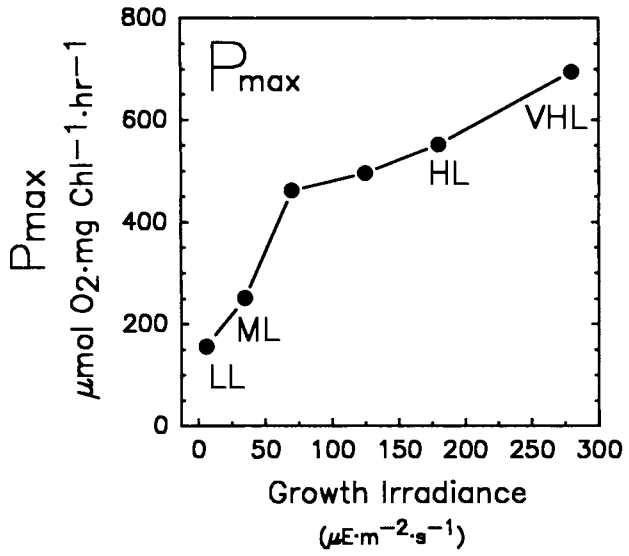

Figure 5. P_{max} per Chl as a function of the growth irradiance. This is not a typical photosynthesis versus irradiance plot.

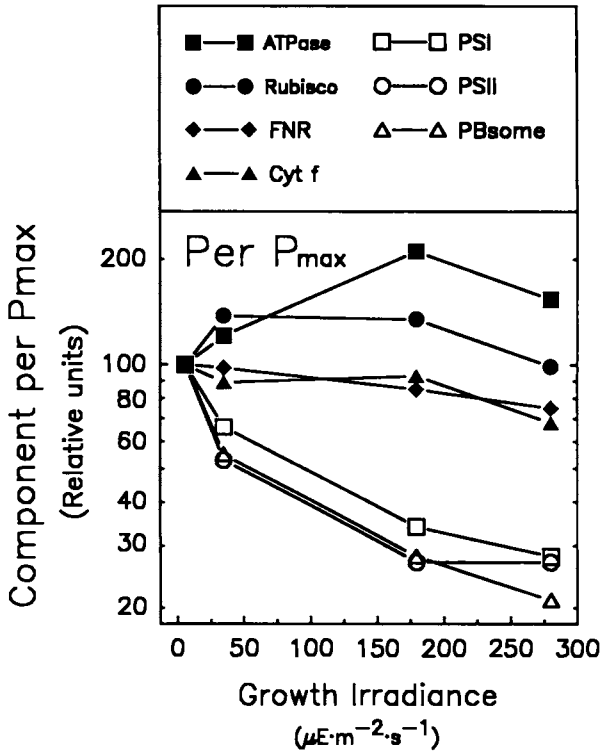

Figure 6. Photosynthetic enzymes and light-harvesting components per P_{max} as a function of the growth irradiance. Data were normalized to a value of 100 for cultures grown under 6 $\mu E \cdot m^{-2} \cdot s^{-1}$.

Enzyme Content versus Photosynthetic Activity

P_{max} in cultures of *P. cruentum* varied directly with the PPFD of the light under which the culture was grown (Fig. 5). In VHL cultures, the rate was more than 4-fold that measured in cultures grown under LL (Fig. 5). To ascertain the biochemical basis for these differences in P_{max} and to identify potential rate-limiting reactions of photosynthesis, we plotted the amounts of various enzymes and light-harvesting components per P_{max} as a function of the growth irradiance (Fig. 6). A horizontal line plot would be indicative of a constant proportionality of a component to P_{max}.

From the plots in Figure 6, it is apparent that numbers of PSI or PSII do not limit photosynthetic electron transport under saturating light in cultures grown under LL or ML, although either photosystem might be limiting in cultures grown under HL or VHL. From the P_{max} values and the numbers of photosystems at each irradiance, we calculate that the minimal turnover times or τ (cf. ref. 36) for PSII and PSI reaction centers are about 19 and 35 ms, respectively, in LL but only about 5 and 10 ms, respectively, in HL- and VHL-grown cultures.

In contrast to the numbers of PSI and PSII centers, the amount of ATPase per P_{max} is at a minimum in LL-grown cultures, with substantially more ATPase per P_{max} in cultures grown under HL. Amounts of Cyt *f*, FNR, and, less strictly, Rubisco are nearly constant in proportion to P_{max} over almost the entire range of irradiance. The calculated minimal turn-

over time for Cyt *f* is about 9 ms for LL-, ML-, and HL-grown cultures.

Acclimation to Change in Spectral Composition of the Growth Light

The light-harvesting antenna systems that serve PSI and PSII in *P. cruentum* have very different, nearly complementary absorption spectra. The green and orange light-absorbing PBsomes are associated with and transfer absorbed light energy to PSII (7, 10), whereas most of the blue and red light-absorbing Chl in *P. cruentum* is associated with PSI (13, 27). Thus, illumination of a WL-grown culture of *P. cruentum* with GL or with RL will initially introduce a severe inequality in the relative number of photons captured by the antenna systems of the two photosystems. In a previous study (13), we observed that in cultures grown for several generations under continuous RL or GL the stoichiometry of the photosystems was altered in such a way as to partially redress this imbalance. We now ask whether levels of Rubisco, ATPase, Cyt *f*, or FNR are also affected by the spectral composition of the growth light, and if so, are the changes in the same direction and/or to the same degree as for PSI or PSII reaction centers.

A comparison of the concentrations of these enzymes and antenna complexes in thylakoid membranes of RL- and GL-grown cultures is presented in Figure 7. The data are given on the basis of thylakoid membrane area, but plots on the basis of Chl or total cell protein are similar (Chl per thylakoid area and total cell protein per thylakoid area in RL membranes are, respectively, about 90 and 110% of values in GL membranes). Numbers of PBsomes per thylakoid surface area were counted directly in electron micrographs of negatively stained spread thylakoid membranes (451 ± 25 PBsomes per μm^2 in RL and 394 ± 40 in GL [33]). From this PBsome data, the absolute or relative densities of the other components within the membrane were calculated.

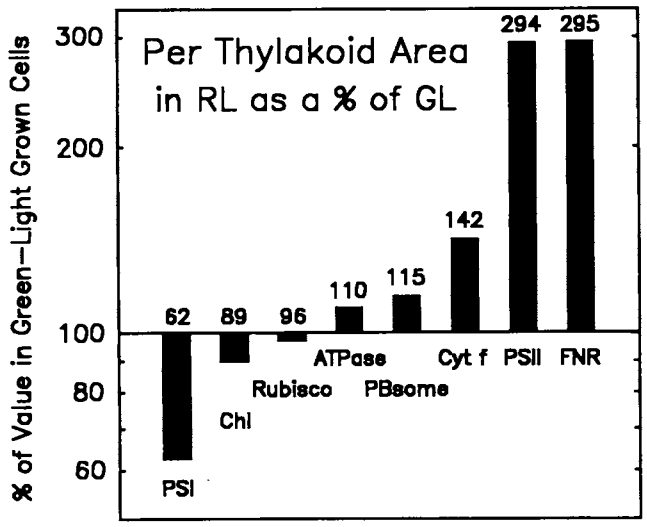

Figure 7. Influence of the spectral composition of the growth light on the concentration of photosynthetic enzymes and light-harvesting components in thylakoid membranes of *P. cruentum*. Cultures were grown under continuous RL or GL of 15 $\mu E \cdot m^{-2} \cdot s^{-1}$. Numbers above the histogram bars indicate the concentration in RL thylakoids as a percentage of the concentration in GL. Numbers of PBsomes per membrane area are from direct counts in electron micrographs. The densities of the other enzymes and components were calculated with reference to these PBsome values. Data for cultures grown under GL were normalized to a value of 100. A normalized value of 100 is equivalent to 4.0×10^5 Chl, 2520 PSI (P_{700}), 630 PSII (Q_A), 540 Cyt *f*, and 390 PBsomes per μm^2 of thylakoid membrane area.

Compared with those grown under GL, cultures of *P. cruentum* grown under RL have 40% fewer PSI centers (P_{700}), 40% more Cyt *f*, and 3-fold as many PSII centers (Q_A) and FNR per unit area of thylakoid membrane (Fig. 7). The concentrations of Rubisco, ATPase, and PBsomes in RL membranes were not much different from those in GL membranes.

The cultures of *P. cruentum* used in this study were grown under equal PPFDs of continuous RL or GL (15 $\mu E \cdot m^{-2} \cdot s^{-1}$) in an attempt to minimize differences in quantum capture by the photosynthetic apparatus. However, because there are differences in the ability of *P. cruentum* to harvest and utilize specific regions of the spectrum, the differences in enzyme content displayed in Figure 7 cannot necessarily be ascribed solely to the chromatic properties of the light. From the data presented earlier (Fig. 3), we know that levels of ATPase and Rubisco per Chl (and per thylakoid area) are more drastically affected by irradiance than are levels of the other enzymes, electron carriers, or light-harvesting components examined. The small differences in the amounts of ATPase and Rubisco per thylakoid area in RL versus GL cultures (Fig. 7) and the comparable growth rates exhibited by cultures grown under RL and GL (not shown) indicate that the more substantial changes observed for other enzymes and components (Fig. 7) are, in fact, due primarily to the difference in the chromatic nature of the two light fields rather than to a difference in irradiance.

DISCUSSION

Irradiance Acclimation and Plasticity of the Thylakoid Membrane

The concept of a plastic photosynthetic apparatus, changing in response to alterations in the light environment to enhance photosynthetic efficiency, is supported by a plethora of studies of photoacclimation in plants, algae, and cyanobacteria (1, 2, 6, 16, 26, 36–38). Acclimation of the red alga *P. cruentum* to changes in irradiance, as in many of these other photosynthetic organisms, involves changes in the numbers of light-harvesting antenna pigments and reaction centers relative to the amounts of enzymes and electron carriers that process and utilize the absorbed quanta. For *P. cruentum*, the concentrations of reaction centers and pigments within the thylakoid membrane are relatively constant, diminishing only slightly under high irradiance. Rather, it is the concentrations of Cyt *f*, FNR, and ATPase and the amount of Rubisco relative to membrane area that vary by as much as 3- or 4- (FNR, Cyt *f*, Rubisco) to 6-fold (ATPase). Such plasticity of the photosynthetic membrane has obvious advantages. The biosynthesis and maintenance of a capacity

for electron transport, CO_2 fixation, or light-harvesting, in excess of what is required or can be utilized under the growth conditions at hand, can exact a heavy metabolic cost. The PBsomes in *P. cruentum*, for example, can account for more than half of the total cell protein in cultures grown under low PPFD (21; F.X. Cunningham and E. Gantt, unpublished data), and Rubisco can comprise a significant proportion of the protein in cells of *P. cruentum* grown under high PPFD (21). Yet, it is also obvious that even a 4- to 7-fold change in the ratio of enzymes and electron carriers to antenna pigments can be only a partial solution when the PPFD is changed by a factor of 50, and this degree of change in *P. cruentum* exceeds that typically observed in other plants, algae, and cyanobacteria examined (1, 2, 18, 29).

In LL-grown cultures of *P. cruentum* the light-saturated rate of photosynthesis is much greater than the rate exhibited under the PPFD to which the culture has been acclimated (not shown). Why do cells of such cultures, allowed to acclimate for more than 4 weeks and many generations, retain a capacity for photosynthesis well in excess of what can be utilized under the light conditions? Why is the membrane not even more plastic than we have observed? Given the diurnal periodicity of light in natural ecosystems, the movement of unicellular algae in the water column and the brief or longer periods of high irradiance that commonly occur even for shaded plants and algae, it may well be of selective advantage to maintain a high capacity for photosynthesis in anticipation of the higher PPFD that is almost sure to follow.

Correlation of P_{max} with Cyt *f*, FNR, Rubisco, and ATPase

Depending on the irradiance under which they are grown, cultures of *P. cruentum* exhibit light-saturated rates of photosynthesis that can differ by a factor of four or more (Fig. 5). Amounts of FNR, Cyt *f*, and Rubisco in *P. cruentum* correlated well with change in P_{max} over most of the broad range of irradiance examined (Fig. 6). Similar observations have been made in the past for Cyt *f*, Rubisco, and/or ATPase in other organisms (1, 2, 6, 11, 16, 26, 36–38), but much less is known about FNR (16). Because of a strong correlation between P_{max} and Cyt *f* in pea, it was earlier suggested that flow of electrons through the Cyt b_6f complex may be a limiting factor in photosynthesis (37). However, a stoichiometric relationship between P_{max} and Cyt *f* is not always observed. In *Dunaliella tertiolecta*, for instance, it was reported that P_{max}, together with Rubisco, increased by a factor of four relative to Cyt *f* and PSII after an increase in the growth irradiance (36). A close correlation in the amounts of Cyt *f* and PSII has also been reported for the cyanobacterium *Synechocystis* PCC 6714 grown under a wide variety of light conditions (18). For *D. tertiolecta*, the suggestion was made that Rubisco is a limiting factor in photosynthesis (36). Woodrow and Mott (38) concluded that Rubisco is the "principal determinant of the photosynthetic rate" under saturating light in *Helianthus annuus* as well. Our data are consistent with either electron transport or CO_2 fixation or both as determinants of the light-saturated rate of photosynthesis in *P. cruentum*.

Chromatic Acclimation

Plots of the irradiance acclimation data (Figs. 3 and 6) illustrate a close correlation between FNR, Cyt *f*, Rubisco, and P_{max} on one hand, and between PSI, PSII, and PBsomes on the other, whereas ATPase varied with irradiance in a third, unique way. With a change in the chromatic composition of the growth light, many of these relationships are no longer maintained (Fig. 7). The variable stoichiometry for PSI, PSII, and PBsomes has been discussed in a previous publication (13). Of particular interest here is the substantial and unexpected increase in the concentration of FNR under RL, even as Rubisco, ATPase, Cyt *f*, and P_{max} (P_{max} in RL cultures is about 20% higher than in GL; data not shown) changed little or increased only moderately (Fig. 7). The 3-fold increase in FNR is accompanied by a comparable increase in the density of PSII centers. If FNR serves solely to mediate the reduction of NADP by Fd, which is itself reduced by PSI, then the amount of enzyme activity required by the cell would be proportional to the maximum rate of linear electron transport. Given the similar levels of Cyt *f* and P_{max} in RL and GL, it is unlikely that this rate is much higher in RL than in GL. Why then a 3-fold increase in FNR under RL? One possibility is that FNR plays a role in cyclic electron transport around PSI. Some evidence for this has been presented in the past (19), and the existence of at least two different pools of FNR, one soluble and the other tightly bound to the thylakoid membrane, supports the idea of two functions for this enzyme. Just where FNR might fit into the cyclic pathway and what are the differences between soluble and membrane-bound FNR remain unclear.

A second explanation for the substantial increase in FNR under RL is suggested by the intriguing report of Arnon and Barber (3) that PSII is capable of reducing FNR in the absence of PSI. If this pathway is operative in *P. cruentum*, then an increase in FNR under RL is of obvious utility and an important aspect of the acclimation process in this organism.

Functional considerations, specifically the high efficiency of photosynthesis under GL absorbed predominantly by PBsomes (7, 27), imply that there must be a close interaction between PBsomes and at least some of the PSI and PSII in thylakoid membranes of *P. cruentum*. There is no fixed ratio between any two of these three complexes, but the total number of eight photosystems (PSI + PSII) per PBsome (13, 14) is unaffected by change of irradiance or spectral composition of the growth light. This constancy, together with the proximity of PSI, PSII, and PBsomes implied by functional studies, suggests a higher level of organization involving these three complexes. We are now pursuing an immunocytochemical approach to physically map individual photosystems on the thylakoid membrane and thereby ascertain the particulars of their association.

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