Plant Physiol. (1984) 74, 451–454
0032-0889/84/74/0451/04/$01.00/0

Short Communication

Effective Absorption Cross-Sections in *Porphyridium cruentum*[1]

IMPLICATIONS FOR ENERGY TRANSFER BETWEEN PHYCOBILISOMES AND PHOTOSYSTEM II REACTION CENTERS

Received for publication October 26, 1983 and in revised form December 7, 1983

ARTHUR C. LEY*
The Rockefeller University, New York City, New York 10021

ABSTRACT

Effective absorption cross-sections for O_2 production by *Porphyridium cruentum* were measured at 546 and 596 nanometers. Although all photosystem II reaction centers are energetically coupled to phycobilisomes, any single phycobilisome acts as antenna for several photosystem II reaction centers. The cross-section measured in state I was 50% larger than that measured in state II.

The phycobiliproteins are major light-harvesting components in the photosynthetic apparatus of the red algae and cyanobacteria (9). In these organisms, the phycobiliproteins are contained in large, highly organized, multi-protein aggregates called PBsomes[2] which are located on the external surface of the photosynthetic membrane (4). Despite their extralamellar location, the PBsomes transfer absorbed light energy with high specificity and efficiency to PSII which is located within the membrane (11, 12). The quantitative relationships between PSII and the PBsomes are still not clear. For example, recent quantitative studies of the RC and pigment content of red algae and cyanobacteria (10, 25, 28) have shown that the RCII:PBsome ratio is variable and can often be greater than unity. Furthermore, Diner (1) Wollman (29) have presented evidence that in the red alga *Cyanidium caldarium* about half of the RCII do not receive any of the light energy absorbed by the PBsomes.

We (14) have recently developed a method for the determination of the effective *in vivo* absorption cross-section of PSII. In this report, we describe the use of this technique to investigate relationships between the absorption of light by the PBsomes and its use for photochemistry by RCII in the red alga *Porphyridium cruentum.*

MATERIALS AND METHODS

Cultures of the unicellular red alga *Porphyridium cruentum* at 20°C were grown in an artificial seawater medium (21) in 250-ml flasks on rotary shaker laterally illuminated with continuous light (8×10^3 erg cm^{-1}) from a bank of cool-white fluorescent lamps. Cells were collected from 5- to 7-d-old exponentially growing cultures by centrifugation (5 min at 10,000*g*) and the pellet was resuspended in fresh growth medium at an equivalent Chl concentration of 10 to 20 μM. We calculate that, after settling on the electrode surface, the cells covered less than 30% of the floor area of our O_2 polarograph (14).

To measure effective absorption cross-sections in *P. cruentum*, I used a modification of our previously published method (14). I simultaneously illuminated cells resting on the surface of the bare platinum electrode with two sources of light. The first was a continuous green (541 nm) or blue (446 nm) background light. The second was a train (0.2 Hz) of laser flashes. To establish state II or state I, the cells were preilluminated for 5 min with bright green (100×10^{14} quanta cm^{-2} s^{-1}) or bright blue (61×10^{14} quanta cm^{-2} s^{-1}) continuous light, respectively. These bright irradiances are at the upper end of the range of irradiances used in previous studies to produce state transitions (13, 19, 22, 24). Preillumination of the algae with bright light gave the most stable and reproducible rates of O_2 evolution in subsequent dim illuminations. The bright green and bright blue illuminations produced rates of O_2 production that were 89% and 28%, respectively, of the light-saturated rate. For the cross-section measurements, we then reduced background irradiances to 1.0×10^{14} quanta cm^{-2} s^{-1} (green) or 1.7×10^{14} quanta cm^{-2} s^{-1} (blue). The dim 541 and 446 nm illuminations supported rates of O_2 production that were 6% and 3% of the light-saturated rate, respectively, and both allowed maximum O_2 flash yields. O_2 flash yields and rates of O_2 production reached their low-light steady state values within 2 min after the change from high to low continuous blue irradiance and in 3 to 4 min following the change from high to low continuous green irradiance. We began our cross-section measuremnts, which took a total of 8 to 12 min to complete, as soon as steady state O_2 flash yields had been established.

I generated 546 and 596 nm laser flashes of 0.5 μs total duration using methanolic solutions of 100 μM Coumarin 540 or 50 μM Rhodamine 590 (Exciton, Dayton, OH), respectively, in a flashlamp-pumped dye laser (DL2100C, Phasar Corp., New Durham, NH). We measured the total energy and relative O_2 yield of each laser flash. For cross-section measurements, we attenuated single laser flashes with calibrated solutions of mixed metal (Cr, Co, Cu, and Ni) sulfates. We calculated relative O_2 flash yields as the ratio of the O_2 yield of the attenuated flash to that of the preceding unattenuated flash.

RESULTS

I have determined effective absorption cross-sections for O_2 production by the red alga *P. cruentum* from measurements of the flash energy dependence of O_2 flash yields. Figure 1 shows plots of the data. The relative O_2 yield of the algae in response

[1] Supported by the United States Department of Agriculture contract number 59-2368-1-759-0.

[2] Abbreviations: PBsome, phycobilisome; RCI, the reaction center of photosystem I; RCII, the reaction center of photosystem II.

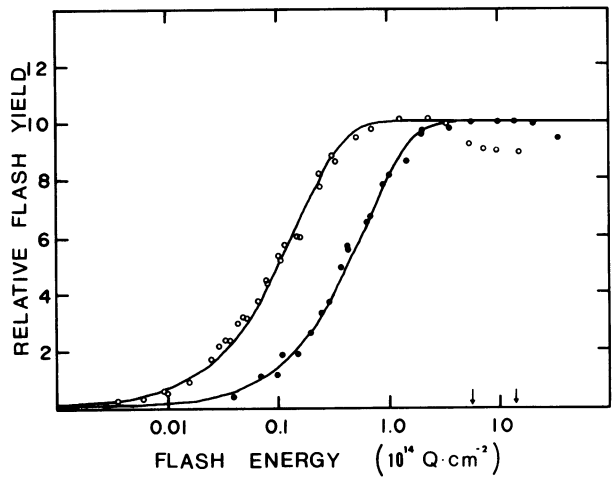

FIG. 1. Light saturation behavior of O_2 flash yields in *P. cruentum*. Relative O_2 flash yields (multiplied by 10) are shown plotted *versus* the energy density of the laser flashes at the electrode surface. (●), Data obtained using 596 nm flashes; (○), data obtained with 546 nm flashes. Both sets of data were collected in the presence of a continuous, dim 541 nm background light. (——), Best fits by eye of the data to the exponential saturation function described in the text. The units for laser flash energies are 10^{14} quanta cm^{-2}.

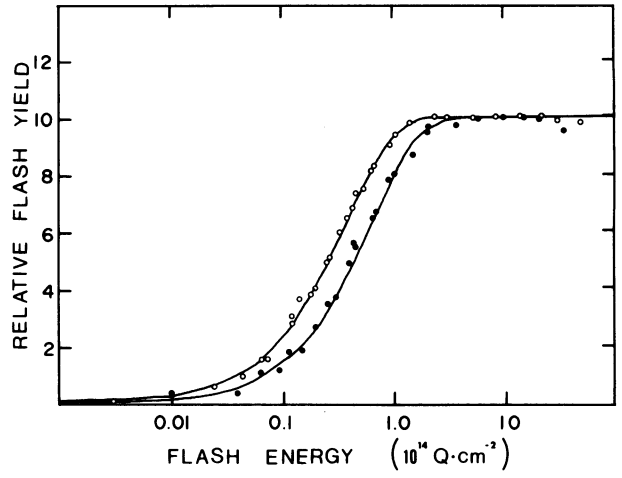

FIG. 2. Effect of background illumination on O_{O_2} at 596 nm. (●), Data obtained in the presence of a continuous dim 541 nm background illumination; (○), data obtained in the presence of a continuous dim 446 nm background illumination. Axes are as in Figure 1.

to a laser flash is shown plotted as a function of the total energy per cm^2 of the flash at the electrode surface. The flash yields shown in Figure 1 were obtained from cells illuminated at either 546 nm (○) of 596 nm (●). The laser flashes were superimposed on the continuous dim 541 nm irradiance as described in "Materials and Methods." At both laser wavelengths, cellular absorption properties are determined primarily by phycobiliprotein and Chl *a* content. However, at 546 nm, absorption by the phycobiliproteins dominates the whole cell absorption spectrum. In contrast, at 596 nm Chl *a* and phycobiliprotein absorptions are comparable.

The two solid curves in Figure 1 are the best fits, by eye, of the simple exponential saturation function (Eq. 1) to the two sets of data:

$$Y = 1\text{-}exp\,(-\sigma_{O_2} E) \quad (1)$$

where Y is the relative flash yield, E is the measured photon flux density at the electrode surface, and σ_{O_2} is the effective absorption cross-section at the laser wavelength for the PSII reaction leading to O_2 production.

Two properties of the data in Figure 1 are worth comment. The first is that, although flash yields are shown on a relative scale, the experimentally measured values for the maximum flash yields were identical for both sets of data. The second is that both sets of data are well described by curves using single values for σ_{O_2}. Effective absorption cross-sections determined from the fits to the data in Figure 1 are 450 $Å^2$ at 546 nm and 160 $Å^2$ at 596 nm.

I found that in *P. cruentum* the wavelength of a continuous background light and the past illumination history of the algae greatly influence the magnitude of σ_{O_2}. Figure 2 shows an example of this effect. I first measured O_2 flash yields from algae simultaneously illuminated with a continuous dim green background light. These data are shown by the closed circles in Figure 2. Next, these algae were illuminated for 5 min with bright blue light and then immediately remeasured O_2 flash yields in the presence of a dim blue background light. The open circles in Figure 2 represent these data. As in Figure 1, both sets of data are well described by curves using single values for σ_{O_2}. However, the value for σ_{O_2} measured in blue light (260 $Å^2$) is 50% greater than that measured in green light (160 $Å^2$).

In addition to changes in σ_{O_2}, the experimentally measured values for the maximum O_2 flash yields were different for the two sets of data shown in Figure 2. Light-saturated O_2 flash yields measured in the presence of a dim green background light, $Y_{max(green)}$, were always smaller than those measured in the presence of a dim blue background light, $Y_{max(blue)}$. The ratio of $Y_{max(green)}$ to $Y_{max(blue)}$ was 0.80. We obtained the same value for $Y_{max(green)}$:$Y_{max(blue)}$ when steady state flash yields were measured from cells which had not been preilluminated with bright green or bright blue light. This decrease in $Y_{max(green)}$ relative to $Y_{max(blue)}$ is qualitatively similar to that reported by Myers *et al.* (20) for O_2 flash yields from cyanobacteria illuminated with light II or light I. In confirmation of their observations, we also find that a saturating flash given during a dim green background illumination results in a transient enhancement of the rate of O_2 production due to the background light. This effect diminishes greatly at subsaturating flash energies and is absent when flashes are superimposed on a continuous blue background illumination. Similar to Myers *et al.* (20), I interpret the decrease in $Y_{max(green)}$ relative to $Y_{max(blue)}$ to reflect an increase in the state of reduction of the plastoquinone pool in green light relative to blue light. It is important to note that the presence of 'closed' RCII does not affect the magnitude of σ_{O_2} determined by the flash saturation technique (14–17).

DISCUSSION

The simple exponential saturation behavior described by Eq. 1 can occur only if the system is completely homogeneous (16, 17). Inhomogeneities arise either from nonuniform illumination conditions (*i.e.* from the light source itself, or from absorption or scattering within the sample) or from heterogeneities inherent to the sample (*i.e.* a distribution of PSII antenna sizes). An important example of the latter occurs when there are two distinct cross-sections present, as would be the case if some RCII were not energetically coupled to PBsomes. The resulting saturation curve would then be the weighted sum of the two individual saturation curves. We estimate that the resolution of our data permits us to distinguish a difference in cross-section of about two if the two kinds of PSII are present in about equal abundance. Large differences in cross-section ($\sigma_1 \geq 4\sigma_2$) result in a 'step-like' saturation curve and are easily distinguished (14, 16, 17).

The saturation curve described by Eq. 1 can also be obtained when RCII share a common antenna, provided excitation energy

arriving at a closed RCII does not escape the reaction center and find another open RCII (16, 17). If 't' RCII share a common antenna of cross-section σ_A, the cross-section determined from the fit of Eq. 1 to the data is $\sigma_{O_2} \simeq \sigma_A/t$, the effective cross-section per RCII (16, 17). If energy arriving at a closed RCII can escape to another RCII, the resulting saturation curve will rise more steeply than that described by Eq. 1, reflecting the increase in cross-section per RCII as reaction centers close (16, 17). (For more detailed discussions of the above points, see Refs. 14–17.)

In light of the above discussion, we conclude from the data shown in Figure 1 that in our *P. cruentum* cells essentially all RCII receive light energy absorbed by the PBsomes. If some RCII were not energetically coupled to PBsomes, I would expect the measured saturation curves to be distinctly bimodal. For example, I calculate that at 596 nm the effective absorption cross-section for RCII not in contact with PBsomes should be between 7 and 18 Å^2. This calculation assumes that between 20 and 50 molecules of Chl *a* act as antenna for RCII (2, 27) and that the *in vivo* absorption cross-section at 596 nm for Chl is about 0.35 Å^2 (14). The arrows in Figure 1 show the laser energies where the '$1/e$ points' (0.63 Y_{max}) of flash saturation curves for O_2 production by RCII with effective absorption cross-sections of 7 or 18 Å^2 would occur. Clearly, there are no significant changes in O_2 flash yields in this range. At 546 nm, the situation would be even more extreme. Inasmuch as I found the same maximum flash yields at both wavelengths, I concluded that the 546 nm measurement did not overlook RCII which do not receive energy from the PBsomes.

The value for σ_{O_2} at 546 nm can be used to estimate the number of PBsomes that act as antenna per RCII. To do so, the absorption cross-section of a *P. cruentum* PBsome must be known. The molar extinction coefficient for B-phycoerythrin, 2.41×10^6 M^{-1} cm^{-1} at 545 nm (7), corresponds to an absorption cross-section of 92 Å^2. Gantt *et al.* (5) estimated that the volume of the *P. cruentum* PBsome is sufficient to contain 70 to 80 molecules of biliprotein. Since phycoerythrin accounts for 84% of the pigments in the PBsome (6), we estimate that about 60 molecules of B-phycoerythrin are contained in the *P. cruentum* PBsome and that the absorption cross-section of the PBsome is about 5500 Å^2 at 546 nm. Our measured value for σ_{O_2} corresponds to about one-seventh of a PBsome acting as antenna per RCII. We conclude that several reaction centers share the light energy absorbed by a single PBsome.

We can easily exclude the possibility that σ_{O_2} at 546 nm has been reduced due to interactions between excited states (15, 17) in the PBsome or CHl antenna of PSII. At the laser energy corresponding to one absorbed photon per RCII ($E = 1/\sigma_{O_2}$), each PBsome has absorbed, on the average, about seven photons during the 500 ns laser flash. The average time between absorbed photons is about 70 ns. This time exceeds the measured lifetime for *in vivo* Chl fluorescence (about 2 ns for closed RCII [8]) by a factor of at least 30. Thus, for these laser energies, at no time is there more than one excitation in the PSII antenna (PBsome plus Chl). Quenchers would have to be formed from single excitations at the expense of PSII photochemistry with the corresponding unacceptably large decrease in the quantum yield for O_2 in continuous dim light. Finally, we note that interactions between excited states do occur at very high laser energies. The decline in O_2 flash yields from their maximum values seen at the highest laser energies (Fig. 1) probably results from the same total annihilation process we have described previously in *Chlorella vulgaris* (15).

The exact number of RCII sharing the light energy absorbed by the PBsome depend not only on the accuracy of our estimate of the *in vivo* absorption cross-section of a PBsome but also on the extent to which light energy absorbed by PBsomes is transferred to PSI. Fluorescence measurements suggest that in *P. cruentum* 30 to 50% of the light energy absorbed by PBsomes is delivered to PSI before it can be used by PSII (11–13). If this is the case, and if the same PBsome functions as antenna for both PSII and PSI, then our result implies that three to four RCII share the light absorbed by a single PBsome.

There are several reports in which stoichiometries between RCII and PBsomes in red algae and cyanobacteria have been estimated from quantitation of reaction center and pigment content (10, 25, 28) or by use of electron microscopy (26, 29). The RCII:PBsome ratio has been found to vary between 1 and 4 (10, 25, 28). RCII:PBsome ratios differ not only among different species but also within the same species, depending on growth conditions. Since I find that all RCII are associated with PBsomes, I can estimate from the data that the RCII:PBsome ratio in *P. cruentum* is 3 to 4 (provided that there is no significant number of PBsomes exclusively associated with PSI). This ratio is similar to that reported by Kursar and Alberte (10) for the macrophytic red alga *Neoagardhiella baillyei* and is consistant with the 'apparent photosynthetic unit size' for *P. cruentum* reported by Mimuro and Fujita (18).

In order to use RCII:PBsome ratios to calculate PSII antenna sizes, one must know the extent to which RCII are connected to PBsomes. While we find that in *P. cruentum* all RCII are coupled to PBsomes, this apparently is not universally true. Diner (1) has reported that in *Cyanidium caldarium* about half of the RCII do not receive light energy from PBsomes.

The effects of background irradiation on σ_{O_2} (Fig. 2) are significant in terms of the state II to state I transition (13, 19, 22, 24). For red algae, state I has been defined as the state obtained after long illumination with light primarily absorbed by PSI (446 nm) while state II is the state reached following long illumination with PSII light (541 nm) (13, 19, 22, 24) or after a long dark interval (22, 24). The transition between state II and state I has been postulated to involve a change in the degree of energy transfer between PSII and PSI (13, 19, 22, 24). Recent experimental results have demonstrated changes in the light-limited rates of characteristic PSII and PSI reactions due to state transitions in intact cells (3, 23, 24) which indicate changes in quantum yields, absorption cross-sections, or both.

The results in Figure 2 are a direct demonstration that in *P. cruentum* the state II to state I transition is accompanied by a large (~50%) increase in the effective absorption cross-section per RCII. The change in σ_{O_2} may be accomplished either by a decrease in the degree of energy trnasfer from PSII to PSI (13) or by the actual physical redistribution of antenna components between PSI and PSII in the cell.

Acknowledgments—The author thanks Drs. D. Mauzerall and J. Barrett for their helpful comments during the preparation of this manuscript.

LITERATURE CITED

1. DINER BA 1979 Energy transfer from the phycobilisomes to photosystem II reaction centers in wild type *Cyanidium caldarium*. Plant Physiol 63: 30–34
2. DINER BA, F-A WOLLMAN 1979 Functional comparison of the photosystem II center-antenna complex of a phycocyanin-less mutant of *Cyanidium caldarium* with that of *Chlorella pyrenoidosa*. Plant Physiol 63: 20–25
3. FORK DC, K SATOH 1983 State I-State II transitions in the thermophilic blue-green alga (Cyanobacterium) *Synechococcus lividus*. Photochem Photobiol 37: 421–427
4. GANTT E 1981 Phycobilisomes. Annu Rev Plant Physiol 32: 327–347
5. GANTT E, CA LIPSCHULTZ, BA ZILINSKAS 1976 Further evidence for a phycobilisome model from selective dissociation, fluorescence emission, immunoprecipitation, and electron microscopy. Biochim Biophys Acta 430: 375–388
6. GANTT E, CA LIPSCHULTZ 1974 Phycobilisomes from *Porphyridium cruentum*: pigment analysis. Biochemistry 13: 2960–2966
7. GLAZER AN, CS HIXSON 1977 Subunit structure and chromophore composition of rhodophytan phycoerythrins. J Biol Chem 252: 33–42
8. HAENEL W, JA NAIRN, P REISBERG, K SAUER 1982 Picosecond fluorescence kinetics and energy transfer in chloroplasts and algae Biochim Biophys Acta 680: 161–173
9. JEFFREY SW 1980 Algal pigment systems. Brookhaven Symp Biol 31: 33–58

10. KURSAR TA, RS ALBERTE 1983 Photosynthetic unit organization in a red alga. Plant Physiol 72: 409–414
11. LEY AC, WL BUTLER 1977 The distribution of excitation energy between photosystem I and photosystem II in *Porphyridium cruentum*. *In* S Miyachi, K Katoh, Y Fujita, K Shibata, eds, Special Edition of Plant and Cell Physiology. Japanese Society of plant Physiologists, Tokyo, pp 33–46
12. LEY AC, WL BUTLER 1980 Effects of chromatic adaptation on the photochemical apparatus of photosynthesis in *Porphyridium cruentum*. Plant Physiol 65: 714–722
13. LEY AC, WL BUTLER 1980 Energy distribution in the photochemical apparatus of *Porphyridium cruentum* in State I and State II. Biochim Biophys Acta 592: 349–363
14. LEY AC, DC MAUZERALL 1982 Absolute absorption cross-sections for photosystem II and the minimum quantum requirement for photosynthesis in *Chlorella vulgaris*. Biochim Biophys Acta 680: 95–106
15. LEY AC, DC MAUZERALL 1982 The reversible decline of oxygen flash yields at high flash energies: Evidence for total annihilation of excitations in photosystem II. Biochim Biophys Acta 680: 174–180
16. MAUZERALL DC 1981 Analysis of energy utilization via multiple excitation. *In* G Akoyunoglou, ed, Proceedings of the Fifth International Congress on Photosynthesis. I. Photophysical Processes-Membrane Energization. Balaban International Science Services, Philadelphia, pp 47–58
17. MAUZERALL DC 1982 Statistical theory of the effect of multiple excitation in photosynthetic systems. *In* R Alfano, ed, Biological Events Probed by Ultrafast Laser Spectroscopy. Academic Press, New York, pp 215–235
18. MIMURO M, Y FUJITA 1978 Excitation energy transfer between pigment system II units in blue-green algae. Biochim Biophys Acta 504: 406–412
19. Murata N 1969 Control of excitation transfer in photosynthesis. T. Light-induced change of chlorophyll *a* fluorescence in *Porphyridium cruentum*. Biochim Biophys Acta 172: 242–251
20. MYERS J, J-R GRAHAM, RT WANG 1983 On the O_2 flash yields of two cyanophytes. Biochim Biophys Acta 722: 281–290
21. PROVASOLI L, JJA MCLAUGHLIN, MR DROOP 1957 The development of artificial media for marine algae. Arch Mikrobiol 25: 392–428
22. RIED A, B REINHARDT 1977 Distribution of excitation energy between photosystem I and photosystem II in red algae. II. Kinetics of the transition between State I and State II. Biochim Biophys Acta 460: 25–35
23. SATOH K, DC FORK 1983 State I-State II transitions in the green alga *Scenedesmus obliquus*. Photochem Photobiol 37: 429–434
24. SATOH K, DC FORK 1983 A new mechanism for adaptation to changes in light intensity and quality in the red alga, *Porphyra perforata*. Biochim Biophys Acta 722: 190–196
25. STEVENS CLR, J MYERS 1976 Characterization of pigment mutants in a blue-green alga *Anacystis nidulans*. J Phycol 12: 99–105
26. STAEHELIN LA, EH GIDDINGS, P BADAMI, WW KRZYMOWSKI 1978 A comparison of the supramolecular architecture of photosynthetic membranes of blue-green, red, and green algae, and of higher plants. *In* DW Dreamer, ed, Light Transducing Membranes. Academic Press, New York, pp 335–355
27. VERNON LP, ER SHAW, T OGAWA, D RAVEED 1971 Structure of photosystem I and photosystem II of plant chloroplasts. Photochem Photobiol 14: 343–357
28. VIERLING E, RS ALBERTE 1980 Functional organization and plasticity of the photosynthetic unit of the cyanobacterium *Anacystis nidulans*. Physiol Plant 50: 93–98
29. WOLLMAN F-A 1979 Ultrastructural comparison of *Cyanidium caldarium* wild type and III-C mutant lacking phycobilisomes. Plant Physiol 63: 375–381