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Functional Comparison of the Photosystem II Center-Antenna Complex of a Phycocyanin-less Mutant of *Cyanidium caldarium* with that of *Chlorella pyrenoidosa* [1]

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BRUCE A. DINER AND FRANCIS-ANDRÉ WOLLMAN
Institut de Biologie Physico-Chimique, 13, rue Pierre et Marie Curie, 75005 Paris, France

ABSTRACT

The photosystem II (PSII) antenna of a phycocyanin-less mutant (III-C) of *Cyanidium caldarium* was studied by means of O_2 activation and fluorescence induction measurements and compared to that of the green alga *Chlorella pyrenoidosa*.

In *Cyanidium* this antenna contains only about 40 (Chl) *a* molecules per center yet shows PSII energy transfer as efficient as in *Chlorella* with 240 Chl *a* + *b* per center. In *Cyanidium*, PSII energy transfer occurs among at least four centers which are located close to each other. *Cyanidium* and *Chlorella* show the same PSII action and low temperature emission spectra, in the Chl *a* region despite the gross differences in antenna size.

It is proposed that the PSII center-antenna complex found in *Cyanidium* is a core unit of the center-antenna complex of green algae; the only major difference between them is the addition of light-harvesting Chl *a*/*b* in the latter. It is further proposed that this core is the site of energy transfer between PSII centers.

Haxo and Blinks (15) demonstrated that Chl *a* was only a minor presence (relative to phycobilin) in the PSII action spectra of red algae, despite Chl concentrations per cell close to that of green algae and higher plants. This observation was an early indication that in these algae, only a small fraction of the total Chl was associated with the PSII antenna, the rest contributing to the antenna of PSI, as shown by Ley and Butler (22) in *Porphyridium cruentum*.

Cyanidium caldarium is a thermophylic, acidophylic alga containing C-phycocyanin as an accessory pigment to Chl *a* in the photosynthetic antenna. This alga does not contain Chl *b* and has been classified by Bogorad (6) as being most likely a Rhodophyte.

A mutant lacking phycobilin (mutant III-C) was isolated by Nichols and Bogorad (25). According to preliminary studies, this organism and a cell-free O_2-evolving preparation derived from it appeared to contain only about 20 Chl/PSII reaction center (11). Because this antenna was much smaller than that in higher plants and green algae (~240 Chl) (13, 28) we wondered whether the antenna of *Cyanidium* was not a basic building block of the latter. We undertook a comparative study of the PSII antenna of the III-C mutant of *Cyanidium* and of *Chlorella pyrenoidosa*.

These experiments concern the structure of the center-antenna unit, and the role of the antenna in energy transfer between units. The following paper (12) will discuss antenna synthesis during greening of *Cyanidium* cells previously grown in the dark. Further communications will concern energy transfer between the phycobilisome and the Chl antenna in wild type *Cyanidium* (10) and freeze-fracture electron microscopy of the mutant and wild type (30).

MATERIALS AND METHODS

Chlorella pyrenoidosa was grown on modified Knop medium (16) containing Arnon's trace elements A_5 and B_6 (4) and illuminated by fluorescent light of about 3,000 lux. Cells maintained at 25 C were continuously bubbled with air containing 5% CO_2 at a flow rate of 300 l/hr.

Cyanidium caldarium mutant III-C and wild type (both obtained from L. Bogorad) were grown in Allen minimal medium (2) at 38 C and continuously bubbled with air containing 5% CO_2 at a flow rate of 100 l/hr. They were illuminated with fluorescent light at an average light intensity of 3,000 lux.

O_2 measurements in flashing or modulated light were performed using an O_2 electrode similar to that described by Joliot and Joliot (20). PSII action spectra were performed in a similar apparatus, built by Bennoun, in which a weak modulated beam (25 Hz) of varying wavelength was superimposed on continuous far red background light preferentially exciting PSI. The modulated beam was passed through a Huet monochrometer (model M25) producing monochromatic light with a bandwidth of 3 nm at half-height. PSI was excited throughout at a rate greater than 10 times that of PSII. The modulated O_2 signal was detected using a PAR JB-6 (Princeton Applied Research) lock-in amplifier.

Fluorescence induction was measured using an apparatus described by Bennoun (5). Exciting light was filtered by blue filters as indicated in the relevant figures. Complementary red blocking filters on the Radiotechnique XP 1002 photomultiplier were two layers each of Kodak Wratten 70 and Ulano Rubylith.

Algal cells were suspended in 50 mM K-phosphate (pH 7.0). KCl (0.1 M) was added for the O_2 measurements. All of the above measurements were performed at 22 C.

Low temperature fluorescence emission spectra were performed in an apparatus built by H. Jupin. Algal cells in their culture media were deposited on Millipore filters and rapidly frozen to 77 K by plunging into liquid N_2. Algal cells were maintained at 77 K and preilluminated to block PSII. Exciting light was filtered by a Schott BG 38. Emitted light was passed through a Bausch & Lomb 250 mm monochrometer (bandwidth 6.6 nm at half-height) and detected by a photomultiplier (Dumont model 6911).

RESULTS

Action Spectra. PSII action spectra of *Chlorella* and of the III-C mutant of *Cyanidium* are shown in Figure 1a. There is an obvious absence of a Chl *b* band at 645 nm in the *Cyanidium*

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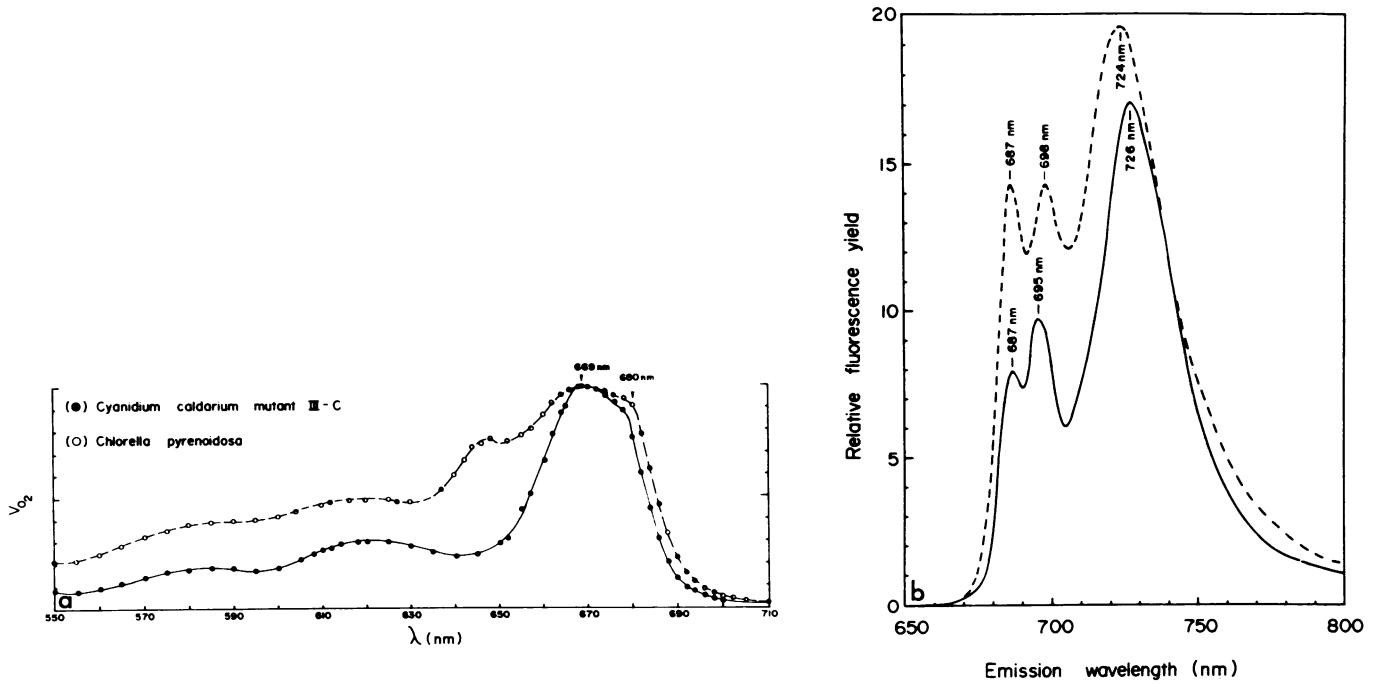

FIG. 1. a: PSII action spectra of *Chlorella* (○) and *Cyanidium* mutant III-C (●). Algae were illuminated with continuous far red light (>680 nm) favoring excitation of PSI relative to PSII, yet sufficient to maintain PSII activated. Weak variable wavelength light (3 nm bandwidth at half-height) chopped at 10 Hz gave rise to a modulated O_2 signal which served as a measure of PSII activity. Photochemical turnover in PSI was maintained greater than 10 times that of PSII. The spectra were corrected for the lamp emission. b: Fluorescence emission spectra of *Cyanidium* mutant III-C (——) and *Chlorella* (- - -) at 77 K. Exciting light was filtered by a Schott BG 38 and emitted light was passed through a monochrometer with 6.6 nm bandwidth at half-height. Algae were preilluminated at 77 K to close the PSII centers before recording the spectra. Spectra were corrected for the spectral response of the photomultiplier.

spectrum. Both algae show Chl *a* absorption bands with maxima at 669 and 680 nm. By comparing the actinic effect of long wavelength light (>680 nm) to that at the maxima, it is apparent that the *Chlorella* spectrum is considerably flattened relative to that of *Cyanidium*. This flattening is due to a screening effect arising from the appreciable absorption of light passing through a single cell, since only a monolayer of cells was deposited on the electrode for both algae. Practically no such flattening occurs for *Cyanidium* (12) because of the lower Chl concentration/cell.

Fluorescence Emission Spectrum. The fluorescence emission spectra of *Cyanidium* III-C and of *Chlorella* at 77 K are shown in Figure 1b. The cells were preilluminated at 77 K to close the PSII centers before recording the spectra. Similar maxima were observed at 687, 695, and 726 nm for *Cyanidium* and at 687, 698, and 724 nm for *Chlorella*. The two shorter wavelength emission bands have been attributed to PSII and the third to PSI (8).

Antenna Size. Joliot (18) has shown that illumination of dark-adapted *Chlorella* induces a sigmoidal increase in the rate of O_2 evolution as a function of time.

In a four-step model for O_2 production, Kok *et al.* (21) proposed that the rate of O_2 production was proportional to the product of the light intensity and the concentration of state S_3 (3 oxidizing equivalents at the O_2-evolving site). In dark-adapted cells the concentration of this state is zero and the O_2-evolving sites are in states S_0 or S_1 (0 and 1 oxidizing equivalent at the O_2-evolving site, respectively). The O_2 rate is thus initially zero and increases in a sigmoidal fashion to a steady-state level, following proportionately the concentration of state S_3. This kinetic curve is known as "O_2 activation" (18).

Since the evolution from the initial states S_0 and S_1 to the steady-state concentration of S_3 is strictly light-limited at intensities falling within the linear region of the light saturation curve (ref. 18 and Fig. 3a), the O_2 activation curve is a justifiable measure of the photochemical rate of PSII. At a given light intensity then, the activation rate (measured as the half-rise time) will be proportional to the optical cross-section of the PSII antenna and to the quantum yield. It will also depend on the initial relative proportions of states S_0 and S_1.

Since we wish to determine the relative optical cross-sections of the PSII antennae in *Chlorella* and *Cyanidium* we must first evaluate the respective quantum yields and ratios of S_0/S_1 of PSII in these algae. This can be done, in part, by comparing the oscillatory pattern of O_2 yields in saturating flashes for previously dark-adapted *Cyanidium* III-C and *Chlorella*. Here every PSII center is excited with each flash. Were every photoact to be transformed into an oxidizing equivalent, the O_2 yield would be expected to oscillate indefinitely producing O_2 whenever a center in state S_3 were excited. This is not the case and the oscillating yields damp out to a steady-state (Fig. 2). This damping was attributed by Forbush *et al.* (14) to a random fraction of centers unable to stabilize oxidizing equivalents on each flash. This fraction, denoted by "miss" parameter, α, is equal to 0.35 for *Cyanidium* and 0.17 for *Chlorella*, calculated from Figure 2, left. The additional "missing" in *Cyanidium* relative to *Chlorella* is completely eliminated by the addition of 6 mM NaN_3 (23). NaN_3 (6 mM) has no effect on the fluorescence induction curve of *Cyanidium* in the presence of DCMU, indicating that this reagent does not modify the antenna itself.

It will be shown under "Discussion" that there are no factors other than "misses" which diminish the photochemical quantum yield in *Cyanidium*. By fitting calculated saturating-flash O_2 sequences to the experimental ones we find that the initial S_0 to S_1 ratio (0.35:0.65) is approximately the same for both algae, despite the somewhat greater damping in *Cyanidium*.

Typical O_2 activation curves at 564 nm for dark-adapted *Chlorella* and *Cyanidium* mutant III-C are shown in Figure 3a. That the O_2-activation kinetic behavior is indeed light-limited is shown by the two *Cyanidium* curves performed at light intensities differ-

ing by a factor of 2.2. The steady-state amplitude and the rise time are 2.2-fold higher and shorter, respectively, in the higher intensity curve.

The activation curve for *Chlorella* (○), the maximum amplitude of which is normalized to that of *Cyanidium* (△), shows an 8.3-fold faster rise time than the *Cyanidium* curve at the same light intensity. If we correct for the greater damping in *Cyanidium* with respect to *Chlorella,* then the *Cyanidium* activation curve should be accelerated by 25%. This is borne out experimentally in that the activation curves for *Cyanidium* are accelerated by 25% upon the addition of NaN_3. We conclude that at 564 nm, the PSII antenna of the III-C mutant of *Cyanidium* is 6.2 times smaller than that of *Chlorella,* or about 40 Chl *a* molecules/PSII reaction center.

The comparison at 564 nm includes Chl *b* which is lacking in *Cyanidium.* Considering the similarity of the Chl *a* action spectra, a comparison at >680 nm should show the respective sizes of the *Cyanidium* antenna compared to that part of the *Chlorella* antenna containing only Chl *a* (~130 Chl *a,* Ref. 28). Such a comparison yields a factor of 4.2 in the activation curves (Fig. 3b) which, when corrected for the additional "missing" in *Cyanidium,* gives a factor of 3.2 (about 40 Chl *a*/reaction center). During intermediate stages of greening and for cells grown on 1% glucose medium, the PSII antenna size of the III-C mutant approaches 30 Chl *a*/center (12).

A comparison (Fig. 3b) of O_2 activation curves for *Cyanidium* III-C mutant and wild type at >680 nm (which does not excite the phycocyanin in the latter) shows that these have the same size Chl *a*-containing antenna in PSII.

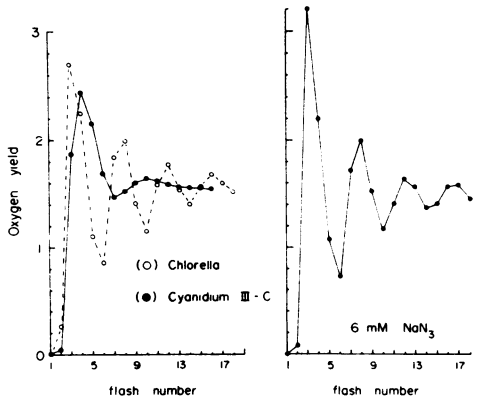

FIG. 2. Left: O_2 yields in a series of saturating flashes given to dark-adapted *Chlorella* (○) and *Cyanidium* mutant III-C (●). Saturating xenon flashes were given every 160 msec to *Chlorella* and every 640 msec to *Cyanidium* III-C after 10 min of dark adaptation. These O_2 flash sequences were best fit by an initial S_0 to S_1 ratio of 0.35:0.65 and "miss" parameters (α) of 0.17 for *Chlorella* and 0.35 for *Cyanidium* III-C. Right: O_2 flash yields in a series of saturating flashes given at 640-msec intervals to dark-adapted (10 min) *Cyanidium* III-C in the presence of 6 mM NaN_3.

Energy Transfer. The shape of the fluorescence induction curve measured in the presence of DCMU is a good indicator of the extent of energy transfer among the centers of PSII (17). Where the center-antenna units are independent and no energy transfer occurs, this induction curve can be described by the following equation which rises exponentially from F_o to F_m (Fig. 4):

$$F = k'i(1 - e^{-kit})$$

where F is the variable fluorescence yield; k and k', rate constants; i, the light intensity; and t, the illumination time. Where the center-antenna units are connected and energy transfer occurs the fluorescence induction curve is sigmoidal in shape. The greater the number of PSII centers that may be visited during the lifetime of an exciton, the more sigmoidal the shape of the fluorescence induction curve.

Figure 4, a and b shows fluorescence induction curves in 10^{-5} M DCMU for dark-adapted *Chlorella* and *Cyanidium* mutant III-C. These show the same sigmoidal shape and a similar ratio of variable to fixed fluorescence yields. The fact that the curves are similar for the two algae suggests that the probability of transfer between their respective units is equivalent. *Cyanidium* wild type (Fig. 4e) shows a somewhat less marked sigmoidal curve and a lower ratio of variable to fixed fluorescence yields than the III-C mutant.

A small fast rise component appears at the start of illumination in the fluorescence induction curves of *Cyanidium* III-C when

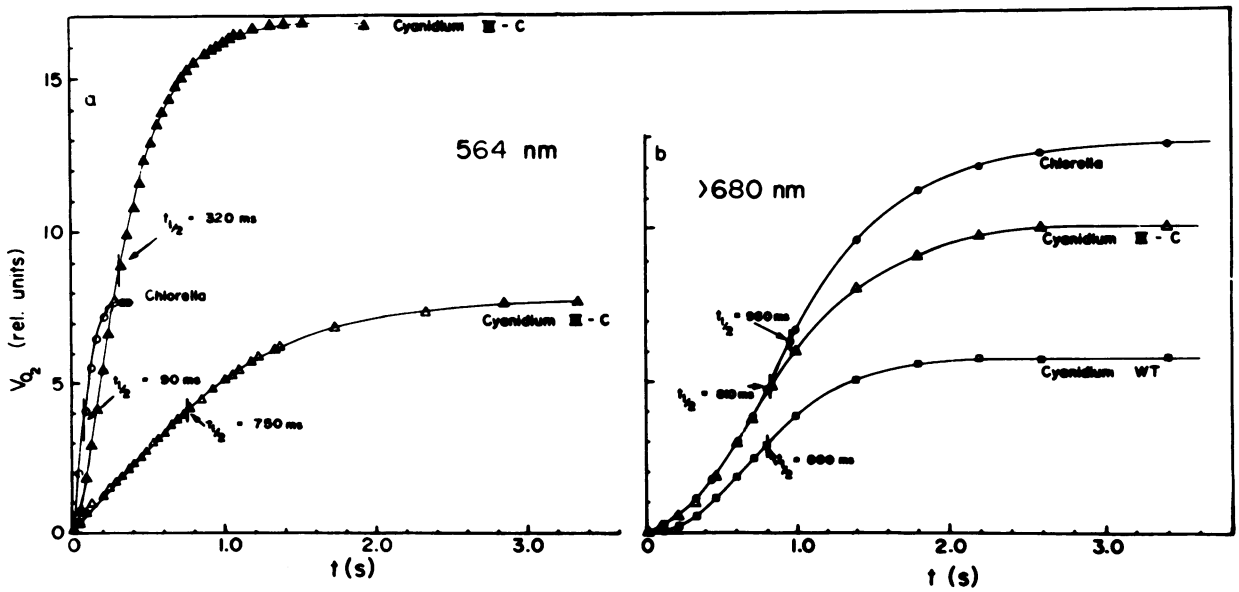

FIG. 3. a: O_2 activation curves at 564 nm of *Cyanidium* III-C (△) and of *Chlorella* (○) at the same light intensity. The full amplitude of the latter curve (○) was normalized to the final rate (V_{O_2}) of the *Cyanidium* curve (△) at the end of the activation. Also shown is an activation curve of *Cyanidium* III-C at 2.2-fold higher light intensity (▲). Exciting light (564 nm, 10 nm bandwidth at half-height) was modulated at 25 Hz and the resulting modulated O_2 rate was detected. Algae were dark-adapted for 10 min and were used at the same cell concentration which was just sufficient to form a monolayer on the platinum electrode surface. b: O_2 activation curves at >680 nm of *Cyanidium* III-C (△) and wild type (□) at the same light intensity and of *Chlorella* (○) at 5-fold lower light intensity. Otherwise the experimental conditions were the same as in Figure 3a.

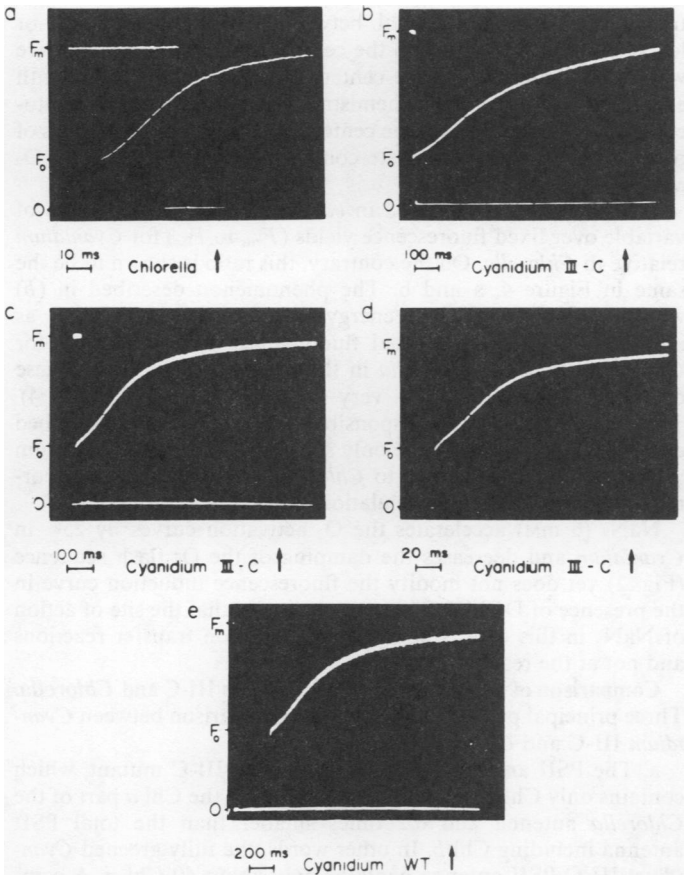

FIG. 4. Fluorescence induction curves of dark-adapted *Chlorella* and *Cyanidium* wild type and mutant III-C in the presence of 10^{-5} M DCMU. Light-driven variation of fluorescence yield (ordinate) is shown as a function of illumination time in msec (abscissa). F_m is the maximum fluorescence yield; F_o, the fluorescence yield at the start of illumination; and 0, the base line with the light off. Only the shapes of these curves are to be compared as the light intensities are not the same. a: *Chlorella* were excited by light filtered by two Schott BG 38 broad band blue filters (each 1 cm thick); b: *Cyanidium* III-C excited using two Schott BG 38 (1 cm each) plus a Seavom 436 nm interference filter (7.5 nm bandwidth at half-height); c: *Cyanidium* III-C excited using one Schott BG 38 (1 cm), one Corning 5-56 plus one Kodak Wratten 32. This combination of filters had a maximum transmission at 460 nm and a 50 nm bandwidth at half-height; d: *Cyanidium* III-C excited using the same filters as in (a); e: *Cyanidium* wild type excited using one Schott BG 38 (1 cm) plus the Seavom 436 nm interference filter used in (b).

broader band-pass light is used. This component is most obvious with the wide band blue Schott BG 38 filters (Fig. 4d), less so with the filter combination of Figure 4c (50 nm bandwidth, 460 nm maximum), and totally absent in Figure 4b (7.5 nm bandwidth, 436 nm maximum). This observation implies the presence of a small fraction of centers attached to an antenna of considerably larger optical cross-section. This additional antenna absorbs to a greater extent in the green than in the blue region of the spectrum. A similar heterogeneity was also apparent in O_2 activation curves, particularly during the first several days of greening (12). As this heterogeneity for the O_2 activation curves was most apparent at 600 nm (maximum ratio of phycocyanin to Chl absorption) and less so at shorter and longer wavelengths, we concluded that the enhanced antenna size was due to the presence of phycocyanin. A comparison of the activation curves at 600 nm for the mutant and the wild type indicated that the phycocyanin component of the antenna (phycobilisome) was the same size in both cases; in the case of the mutant only 1 to 2% of the PSII centers were attached to phycobilisomes. This slight heterogeneity explains why the O_2 activation curves were more sigmoidal (homogeneous) at >680 nm than at 564 nm where phycocyanin is still excited (Fig. 3).

Although Nichols and Bogorad (25) reported the absence of phycocyanin in the III-C mutant, the small quantities reported here may have been below their level of detection. Successive recloning produced colonies which always showed this same slight heterogeneity, as did stock cultures of the same mutant obtained from other laboratories.

Estimate of Number of Interconnected PSII Center-Antenna Units. To determine the number of PSII center-antenna units capable of transferring light energy between them, we blocked an increasing fraction of centers in a low quenching state using DCMU, so that arriving excitons would be transferred with high efficiency to adjacent functional centers. Measuring the resulting optical cross-section for the still functional units would then indicate how many blocked units had contributed their excitons. By measuring the PSII photochemical rate at constant light intensity as a function of the fraction of centers blocked we determined the maximum possible optical cross-section relative to the case where all centers were operating.

State S_3 of the O_2-evolving site was produced at two times its steady-state concentration by exciting dark-adapted algae with two saturating flashes. These were followed by a long series of nonsaturating flashes which drove the concentration of S_3 to its steady-state level in a light-driven process. This experiment is the inverse of the O_2 activation described earlier in that the concentration of S_3 decreases with nonsaturating flash excitation instead of increasing. However, as in these earlier experiments, the kinetic behavior of the relaxation of S_3 to the steady-state was strictly light-limited. Since the yield of O_2 production is proportional to the concentration of S_3, the latter could be followed by determining the O_2 yield of nonsaturating flashes. The photochemical rate mentioned in the previous paragraph is thus the rate of decrease of the O_2 flash yield of nonsaturating flashes following excitation by two saturating flashes.

Dark-adapted *Cyanidium* III-C were given two saturating flashes 300 msec apart producing primarily state S_3. Sixty msec later a series of nonsaturating actinic flashes were given at 60-msec intervals. The O_2 yields of these nonsaturating flashes decrease to a steady-state level about half of that observed on the first flash (Fig. 5). Partially inhibiting concentrations of DCMU were added in the dark period preceding excitation by the two saturating flashes. The rate of decrease of the O_2 flash yields was accelerated upon addition of DCMU because units blocked by this inhibitor were closed by the two-saturating-flash preillumination and transferred their absorbed light energy to adjacent open units in the nonsaturating flash excitation that followed. The intensity of the nonsaturating flashes was maintained constant throughout the experiment.

Increasing the DCMU concentration should tend toward a limiting situation in which an open unit would be surrounded by closed units, producing a maximum increase in the effective optical cross-section of the open unit. Relative to uninhibited cells, those in the presence of 6×10^{-7} M DCMU (11% of the uninhibited O_2 flash yield with nonsaturating flashes) were accelerated by a factor of 3.3. Extrapolating to 100% inhibition (Fig. 6) gave an approximate 4-fold increase in the apparent antenna size, indicating that energy transfer occurs among at least 4 units. This is a minimum estimate as the probability of energy transfer from a closed to an open unit is probably less than one.

NaN_3 (6 mM) was added in the experiment shown in Figures 5 and 6 to assure a greater contrast between the S_3 concentration after two flashes and that in the steady-state.

DISCUSSION

Quantum Yield of Photochemistry. The results concerning the antenna size were interpreted by assuming that the only factor

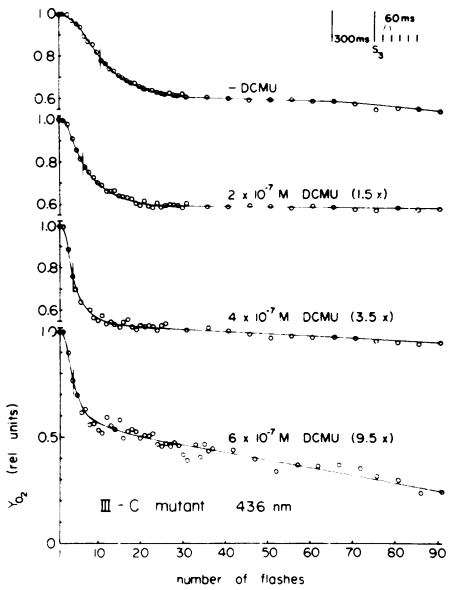

FIG. 5. Flash-driven decrease of the O_2 yield (Y_{O_2}) in a series of nonsaturating flashes following excitation by two saturating flashes in the presence of varying concentrations of DCMU. These curves correspond to the light-driven relaxation of state S_3 to the steady-state level after formation in excess by two saturating flashes. Dark-adapted (10-min) *Cyanidium* III-C were excited by two saturating flashes (300 msec apart) followed 60 msec later by a series of nonsaturating flashes (436 nm, bandwidth at half-height 7.5 nm) given at 60-msec intervals. DCMU was added at the indicated concentrations. Relaxation curves were normalized to the same initial value by multiplying by the indicated factors. NaN_3 (5 mM) was present throughout. Aside from the change in amplitude DCMU does not change the form of the oscillating pattern of O_2 yields in a sequence of saturating flashes.

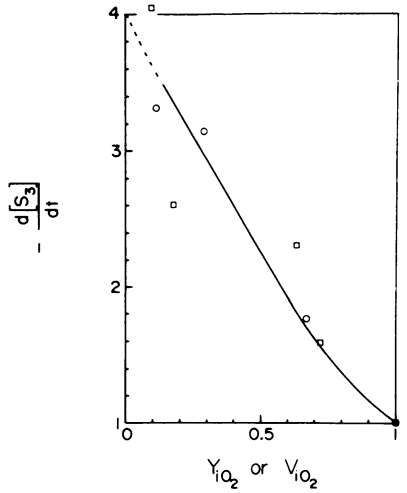

FIG. 6. Relative rate of light-driven decay ($-dS_3/dt$) of excess state S_3 to the steady-state level in either weak flashes (○, 436 nm, data of Fig. 5) or weak modulated light (□, also at 436 nm) as a function of Y_{iO_2} or V_{iO_2} (the relative nonsaturating flash yield or rate of O_2 production at various concentrations of DCMU compared to the uninhibited yield or rate). Y_{iO_2} and V_{iO_2} were measured 60 msec after two saturating flashes were given to dark-adapted cells (Fig. 5). Experimental conditions were as in Figure 5 except in experiment (□) where weak modulated light (25 Hz, 436 nm) was used.

decreasing the quantum yield of photochemistry in *Cyanidium* relative to *Chlorella* was the "misses" (14). That this is probably the case is supported by the arguments given below.

The quantum yield for *Cyanidium* would be lower than that for *Chlorella:* (*a*) if in *Cyanidium* there were less efficient energy transfer between antenna Chl, between the antenna and trap, or less efficient trapping within the center; (*b*) if in *Cyanidium* there were a quencher within the center or antenna competitive with energy trapping for photochemistry; (*c*) if in *Cyanidium* photochemistry occurred within the center but was followed by a loss of oxidizing equivalents at a rate comparable to the kinetics of O_2 activation.

The phenomena described in (*a*) would decrease the ratio of variable over fixed fluorescence yields (F_{var} to F_{fix}) for *Cyanidium* relative to *Chlorella.* On the contrary, this ratio is shown to be the same in Figure 4, a and b. The phenomenon described in (*b*) would show less efficient energy transfer between centers as indicated by a less sigmoidal fluorescence induction curve for *Cyanidium* than for *Chlorella* in the presence of DCMU. These curves are, on the contrary, very similar to each other (Fig. 4). Thus, phenomenon (*c*), responsible for the "misses" described earlier, is most probably the only source of a decreased quantum yield in *Cyanidium* relative to *Chlorella.* The corresponding correction was made in the calculation of the antenna size.

NaN_3 (6 mM) accelerates the O_2 activation curves by 25% in *Cyanidium* and decreases the damping of the O_2 flash sequence (Fig. 2) yet does not modify the fluorescence induction curve in the presence of DCMU. These results imply that the site of action of NaN_3 in this case is on secondary electron transfer reactions and not at the reaction center.

Comparison of PSII Antenna of *Cyanidium* III-C and *Chlorella.* Three principal points characterize the comparison between *Cyanidium* III-C and *Chlorella:*

a. The PSII antenna of the fully greened III-C mutant, which contains only Chl *a*, is 3.2 times smaller than the Chl *a* part of the *Chlorella* antenna and 6.2 times smaller than the total PSII antenna including Chl *b*. In other words, the fully greened *Cyanidium* III-C PSII antenna contains only about 40 Chl *a*. A comparison of the O_2 activation curves at >680 nm (which does not excite phycocyanin) for the III-C mutant and the wild type indicates that they have the same PSII Chl antenna size (Fig. 3b). Partially greened (72-hr) mutant III-C cells show O_2 activation curves slower than both fully greened and less fully greened cells (12). These results probably correspond to a PSII antenna size approaching 30 Chl *a*. A PSII antenna of similar size is obtained with cells grown in the light on Allen medium (2) supplemented with 1% glucose.

b. Despite the small PSII antenna size of the fully greened III-C mutant, these cells show a probability of energy transfer between centers as elevated as that for *Chlorella* (Fig. 4). There is little difference observed in the fluorescence induction curves for cells greened for 72 hr (12) and fully greened cells. Efficient energy transfer can thus occur between PSII antenna consisting of only 30 to 35 Chl.

The massive amounts of Chl associated with the PSII antenna in *Chlorella* are not essential to the mechanism of energy transfer. As such energy transfer requires contact between the Chl surrounding each center, a PSII antenna of 30 Chl necessarily implies that the inter-center distance is quite small in *Cyanidium.* At least four centers are capable of transferring energy between them in this alga. Using a different technique, Joliot *et al.* (19) showed that at least three centers were interconnected in a mutant of the green alga, *Chlamydomonas.*

c. The PSII antenna of *Cyanidium* III-C and *Chlorella* contain the same Chl *a* absorption bands at 669 and 680 nm. These appear to be present in the same relative concentrations in both algae.

The fluorescence emission band at 687 nm has been attributed to the light-harvesting Chl *a/b* complex and that at 695 (698) nm to the reaction center complex Chl a_{II} (8). The fact that *Cyanidium* emits at 687 nm yet lacks the light-harvesting complex casts some doubt on the assignment of this emission band. The ratio of fluorescence yields 687 nm/698 nm is only slightly lower in *Cyanidium* than in *Chlorella.* As their respective antenna sizes

differ by a factor 6.2 principally due to the absence of the light-harvesting Chl *a/b* complex in *Cyanidium*, we conclude that this component of the antenna is at best responsible for only a small fraction of the 687 nm emission in *Chlorella*.

The arrangement and location of the various absorbing components that comprise the PSII antenna are unknown. A concentric layered structure has been proposed with shorter wavelength pigments on the outside and longer wavelength pigments on the inside surrounding the trap (7). This arrangement favors energy migration toward the reaction center. If such a concentric arrangement existed in PSII, we would expect that PSII action spectrum of *Cyanidium* would be red shifted with respect to that of *Chlorella* and equivalent to the interior of the PSII antenna of the latter. Figure 1 shows that this is not the case and that as far as Chl *a* is concerned the pigment composition of their PSII antennae is the same.

There remain, however, three arguments that lead us to believe that there exists a "core" unit of the *Cyanidium* type in algae containing the light-harvesting Chl *a/b* complex.

a. The similarity of the fluorescence emission spectra at 77 K of *Chlorella* and *Cyanidium* III-C: both emit at similar wavelengths (687 and 695 nm) suggesting that in both algae light energy winds up at the same sites.

b. The similarity of the fluorescence induction curves: the absence of a large light-harvesting Chl *a/b* complex in *Cyanidium* III-C does not diminish the efficiency of energy transfer between centers with respect to *Chlorella*. This observation is consistent with the idea that the basic structure (core) necessary for energy transfer has remained intact in *Cyanidium*.

c. One of us (Wollman [30]) has shown through freeze-fracture electron microscopy that particles of about 100 Å are present on the EF fracture face of the thylakoid membranes in *Cyanidium*. Ultrastructural studies of green algae and higher plants which have lost Chl *b* through mutation or intermittent light greening, also show 80 to 100 Å particles on the EF fracture (3). These particles have been attributed to the PSII reaction center plus a small antenna of Chl *a* (a "core" unit) and increase to 160 Å when the Chl *a/b* light-harvesting complex is present (3, 24, 26, 27).

We propose that the PSII center-antenna complex found in *Cyanidium* is a "core" unit of the center-antenna complex of *Chlorella;* the only major difference between them is the addition of the light-harvesting Chl *a/b* complex in the latter. We further propose that this "core" is the site of energy transfer between PSII centers.

Butler and Strasser (9) proposed that the light-harvesting Chl was the pathway for energy transfer in PSII. In our opinion the predictions made by their model are also consistent with the core unit energy transfer that we propose here.

Further published data consistent with a "core" unit of Chl *a* are detailed below.

Akoyunoglou (1) has recently reported that etiolated plastids of bean leaves, when greened for 81 light-dark cycles (2 min light-98 min dark), contain PSII antennae at least five times smaller than in fully greened plastids but which nonetheless allow energy transfer between centers. These small units contain very little Chl *b* (Chl *a*/Chl *b* = 12). He further proposed that greening occurs via early synthesis of PSII "core" units containing active centers and few Chl.

Blue-green and red algae show PSII energy transfer analogous to that observed in *Cyanidium* (via a core of Chl *a*). This core probably corresponds to only a small number of Chl *a* in these algae. Ley and Butler (22) have shown that only 5% of the chlorophyll *a* of *Porphyridium* is associated with PSII. The phycobilisomes do not constitute a pathway for inter-center energy transfer because the phycobilisomes are not in physical contact with one another and energy transfer uphill from Chl *a* to phycocyanin or phycoerythrin is extremely unfavorable. As such core transfer appears to be a generalized phenomenon in the red and blue-green algae, it is likely that a similar arrangement exists in green algae and higher plants.

Based on studies of PSII particles isolated with detergent, Vernon *et al.* (29) proposed the existence of a reaction-center complex containing 15 Chl *a*.

A study of PSII energy transfer and of PSII action and emission spectra, measured under conditions which vary the amount of light-harvesting Chl (mutation, extraction, or intermittent greening), should provide a further test of the "core" model proposed here. Experiments related to energy transfer should be interpreted cautiously, however, as a decrease in the PSII center density alone results in decreased inter-center transfer.

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LITERATURE CITED

1. AKOYUNOGLOU G 1977 Development of the photosystem II unit in plastids of bean leaves greened in periodic light. Arch Biochem Biophys 183: 571–580
2. ALLEN MB 1959 Studies with *Cyanidium caldarium:* an anomalously pigmented chlorophyte. Arch Microbiol 32: 270–277
3. ARMOND PA, LA STAEHELIN, CJ ARNTZEN 1977 Spatial relationship of photosystem I, photosystem II and the light-harvesting complex in chloroplast membranes. J Cell Biol 73: 400–418
4. ARNON DI 1938 Microelements in culture-solution experiments with higher plants. Am J Bot 25: 322–325
5. BENNOUN P 1971 Contribution à l'étude de la réaction photochimique II des organismes photosynthétiques. Thesis, Université de Paris
6. BOGORAD L 1975 Phycobiliproteins and complementary chromatic adaptation. Annu Rev Plant Physiol 26: 369–401
7. BORISOV, A YU 1967 Theory of energy resonance migration in pigment complexes of photosynthetic organisms. Biofizika 12: 630–636 (English translation p. 727)
8. BUTLER WL, M KITAJIMA 1974 A tripartite model for chloroplast fluorescence. *In* M Avron, ed, Proc Third Int Congress on Photosynthesis, Elsevier, Amsterdam, pp 13–24
9. BUTLER WL, RJ STRASSER 1977 Tripartite model for the photochemical apparatus of green plant photosynthesis. Proc Nat Acad Sci USA 74: 3382–3385
10. DINER BA 1979 Energy transfer from the phycobilisomes to photosystem II reaction centers in wild type *Cyanidium caldarium*. Plant Physiol 63: 30–34
11. DINER BA, DC MAUZERALL 1973 Photosynthetic oxygen production and the size of the photosynthetic unit in a cell-free preparation from *Cyanidium caldarium*. Biochim Biophys Acta 292: 285–290
12. DINER BA, FA WOLLMAN 1979 Biosynthesis of photosystem II reaction centers, antenna and plastoquinone pool in greening cells of *Cyanidium caldarium* mutant III-C. Plant Physiol 63: 26–29
13. EMERSON R, W ARNOLD 1932 The photochemical reaction in photosynthesis. J Gen Physiol 16: 191–205
14. FORBUSH B, B KOK, M MCGLOIN 1971 Cooperation of charges in photosynthetic O_2 evolution. II. Damping of flash yield oscillation, deactivation. Photochem Photobiol 14: 307–321
15. HAXO FT, LR BLINKS 1950 Photosynthetic action spectra of marine algae. J Gen Physiol 33: 389–422
16. JOLIOT AP 1960 Contribution a l'étude des phénomènes d'induction de la photosynthèse. Thesis, Université de Paris
17. JOLIOT A, P JOLIOT 1964 Etude cinétique de la réaction photochimique libérant l'oxygène au cours de la photosynthèse. CR Acad Sc Paris 258: 4622–4625
18. JOLIOT P 1968 Kinetic studies of photosystem II in photosynthesis. Photochem Photobiol 8: 451–463
19. JOLIOT P, P BENNOUN, A JOLIOT 1973 New evidence supporting energy transfer between photosynthetic units. Biochim Biophys Acta 305: 317–328
20. JOLIOT P, A JOLIOT 1968 A polarographic method for detection of oxygen production and reduction of Hill reagents by isolated chloroplasts. Biochim Biophys Acta 153: 625–634
21. KOK B, B FORBUSH, M MCGLOIN 1970 Cooperation of charges in photosynthetic O_2 evolution. I. A linear four step mechanism. Photochem Photobiol 11: 457–475
22. LEY AC, WL BUTLER 1976 Efficiency of energy transfer from photosystem II to photosystem I in *Porphyridium cruentum*. Proc Nat Acad Sci USA 73: 3957–3960
23. MAISON-PETERI B, AL ETIENNE 1977 Effects of sodium azide on photosystem II of *Chlorella pyrenoidosa*. Biochim Biophys Acta 459: 10–19
24. MILLER KR 1976 A particle spanning the photosynthetic membrane. J Ultrastruc 54: 159–167
25. NICHOLS KE, L BOGORAD 1960 Studies of phycobilin formation with mutants of *Cyanidium caldarium*. Nature 188: 870–872
26. PARK RB, AO PFEIFHOFER 1969 Ultrastructural observation of deep-etched thylakoids. J Cell Sci 5: 299–311
27. STAEHELIN LA, PA ARMOND, KR MILLER 1977 Chloroplast membrane organization at the supramolecular level and its functional implications. Brookhaven Symp Biol 28: 278–315
28. THORNBER JP, RS ALBERTE, FA HUNTER, JA SHIOZAWA, KS KAN 1977 The organization of chlorophyll in the plant photosynthetic unit. Brookhaven Symp Biol 28: 132–148
29. VERNON LP, ER SHAW, T OGAWA, D RAVEED 1971 Structure of photosystem I and photosystem II of plant chloroplasts. Photochem Photobiol 14: 343–357
30. WOLLMAN FA 1979 Ultrastructural comparison of *Cyanidium caldarium* wild type and III-C mutant lacking phycobilisomes. Plant Physiol. In press