Biochimica et Biophysica Acta, 680 (1982) 95–106
Elsevier Biomedical Press

BBA 41048

ABSOLUTE ABSORPTION CROSS-SECTIONS FOR PHOTOSYSTEM II AND THE MINIMUM QUANTUM REQUIREMENT FOR PHOTOSYNTHESIS IN *CHLORELLA VULGARIS*

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(Received October 12th, 1981)

Key words: Reaction center; Oxygen evolution; Photosystem II; Absorption cross-section; (Chlorella vulgaris)

Absolute absorption cross-sections for oxygen production (σ_{O_2}) were determined from the light-saturation behavior of oxygen flash yields from whole cells of *Chlorella vulgaris* illuminated with submicrosecond flashes of laser light. Light-saturation curves were well described by simple Poisson statistics with a single average cross-section per photosystem trap (RCII). The maximum variation about the average cross-section permitted by the data was a factor of 3. σ_{O_2} at the laser wavelength (596 nm) increased from 38 Å^2 for cells grown in high light to 115 Å^2 for cells grown in low light. The 3-fold variation in σ_{O_2} was accompanied by a 10-fold variation in total cell chlorophyll content. This behavior results, at least in part, from the partitioning of chlorophyll between Photosystem II (measured) and Photosystem I (unmeasured). The 596 nm in vivo absorption cross-section for chlorophyll in *Chlorella* (σ_{Chl}) is 0.29 Å^2, independent of total cell chlorophyll content. The antenna size of RCII was calculated to range from 130 to 400 molecules of chlorophyll. At low flash energies, the relationship between the quantum requirement for oxygen production (QR), the maximum oxygen-flash yield or Emerson and Arnold number (PSU_{O_2}) and our cross-sections is $QR=(PSU_{O_2})\cdot(\sigma_{Chl})/(\sigma_{O_2})$. QR, found to be independent of both total cell pigmentation and RCII antenna size, was constant at 10 ± 1 photons absorbed per oxygen molecule evolved.

Introduction

The pioneering experiments of Emerson and Arnold [1] and Arnold and Kohn [2] and the calculations of Gaffron and Wohl [3] provided the original evidence for the in vivo organization of chlorophyll into large cooperative absorption units. The latter authors proposed that light energy absorbed anywhere within a 'unit' consisting of about 2000 chlorophyll molecules could be used at a single 'center' for the photochemical reactions of photosynthesis. It was recognized that the 'units' were defined in terms of the final products of a possibly multi-staged reaction sequence and so could represent combinations of smaller assemblages. In 1936, Kohn [4] presented evidence for a smaller absorption unit and proposed, in agreement with the then accepted quantum requirement for CO_2 reduction, that four of the smaller units comprised the larger unit of Gaffron and Wohl. One result of the subsequent half-century of research on photosynthesis has been the identification of the major components of the photosynthetic 'unit': the reaction centers of Photosystem I (PS I) and Photosystem II (PS II)—here called RCI and RCII, respectively—and pigment antennae. However, to date, the in vivo size of the pigment antenna absorbing light for either RCI or

Abbreviations: PS I, Photosystem I; PS II, Photosystem II; RCI, the reaction center of Photosystem I; RCII, the reaction center of Photosystem II; Chl, chlorophyll; Y_{O_2}, relative oxygen flash yield.

RCII (the RCI antenna or the RCII antenna) has not been directly measured.

Estimates of reaction center antenna sizes are usually obtained from measurements of 'photosynthetic unit' sizes. The size of a photosynthetic unit was first defined functionally from flashing-light experiments as the ratio of chlorophyll molecules to oxygen molecules evolved per flash when the chloroplast pigments are excited with a long train of saturating, single-turnover flashes of light [5,6]. We shall call this ratio the photosynthetic size for O_2 production: PSU_{O_2}. Recently, photosynthetic unit sizes have been measured as the molar ratio of total chlorophyll to RCI estimated from the absorption changes which accompany the oxidation of P-700 (PSU_{700}) [7–10]. A photosynthetic unit size in terms of Chl/RCII based on the absorption change at 325 nm due to the reduction of the electron acceptor of PS II, Q, has also been reported [10]. Since photosynthetic unit sizes are simple ratios of total chlorophyll to some measured quantity related to reaction center photochemistry (O_2 production, P-700 oxidation, Q reduction), they can be related to effective reaction center antenna sizes only through the use of specific assumptions concerning size and ratio of RCII to RCI. PSU_{O_2} values have been converted to RCII antenna sizes by dividing by 4 (the number of electrons needed to be removed from water to form oxygen), by 8 (assuming the Z scheme and equal numbers of RCI and RCII), or by 10 (the measured quantum requirement for O_2 formation [11]). We will prove that the latter is the correct number to be used. Using this number, the average PSU_{O_2} of *Chlorella* reported by Emerson and Arnold [1], 2480 Chl/O_2, is calculated to yield a RCII antenna size of 250 Chl/RCII.

Relative absorption cross-sections for PS I and PS II in intact cells have been determined by Myers and co-workers [12–14] and Reid et al. [15] from rates of O_2 production; by Butler and coworkers [16–18] from low-temperature fluorescence yield measurements; and by Mimuro and Fujita [19] from measurements of delayed fluorescence. These results show the gross distribution of absorption between the total pigment pools serving both photoreactions but do not directly provide information on the RCI and RCII antenna sizes.

Measurements of the reaction center-related absorbance changes or oxygen flash yields can be used to count the number of reaction centers in a cell. The accuracy of the overall calculation is critically dependent on the accuracies of the assumptions and calibrations involved (e.g., assumed O_2/RC per flash values, difference extinction coefficients, path lengths). Mimuro and Fujita [19] and Myers et al. [14] have used such calculations in conjunction with their measurements of the relative absorption cross-sections of PS I and PS II to determine the size of the chlorophyll antenna for RCII and RCI in blue-green algae. Mimuro and Fujita [19] calculated that in *Anabaena variabilis* Chl/RCI $\approx$ 130 and Chl/RCII $\approx$ 20. Myers et al. [14] found that in several pigment mutants of *Anacystis nidulans*, Chl/RCI $\approx$ 120 and Chl/RCII $\approx$ 50.

In this report we describe the measurement of the absolute physical size of the antenna absorbing light for use by RCII in *Chlorella*. The data analysis is based on the Poisson distribution for hits on effectively isotropic targets. For the simplest assumptions (no escape from open or closed traps) it has previously been shown [20,21] that the measurement determines the average absorption cross-section per trap. This cross-section, σ_{O_2}, is related to the number of chlorophyll antenna molecules by use of the measured in vivo absorption cross-section for a single chlorophyll molecule, σ_{Chl}. Finally, σ_{O_2}, σ_{Chl}, and PSU_{O_2} measurements are used to determine the minimum quantum requirement for O_2 production in flashing light.

Materials and Methods

Axenic cultures of the green alga *Chlorella vulgaris*, Beijerinck, Trelease strain were grown with continuous lateral illumination at 20°C in 500 ml flasks containing 100 ml of a defined mineral liquid medium [22]. Cultures grown in the low light ($(1–2) \cdot 10^2$ erg $\cdot$ cm$^{-2} \cdot$ s^{-1} from cool-white fluorescent lamps) and high light ($(2–3) \cdot 10^5$ erg $\cdot$ cm$^{-2} \cdot$ s^{-1} from a General Electric-Lucolux metal arc lamp) fields were magnetically stirred. Cultures were grown at the intermediate light level ($8 \cdot 10^3$ erg $\cdot$ cm$^{-2} \cdot$ s^{-1} from cool-white fluorescent lamps) on a rotary shaker. Cells were collected from exponentially growing cultures by

centrifugation for 10 min at $10000 \times g$. For use on the O_2 polarograph, pelleted cells were resuspended in a buffered electrolyte solution (0.1 M KH_2PO_4/0.05 M NaCl/0.05 M $NaHCO_3$/0.01 M KCl, pH 7.8). For use with the Clark-type electrode or for whole cell absorption measurements, pelleted cells were resuspended in fresh growth medium with or without 10 mM $NaHCO_3$, respectively.

Chlorophyll concentrations were determined from the absorbance at 665 nm and 649 nm of ethanol extracts of the cells and the equations of Wintermans and De Mots [23]. Cell counts were made in quadruplicate with a hemocytometer.

Apparent absorption spectra for suspensions of *Chlorella* cells were measured with a Cary Model 14 spectrophotometer equipped with a scattered transmission accessory. The reference cuvette contained a suspension of polystyrene particles which we found to mimic the scattering properties of *Chlorella* cells. The concentration of the reference suspension was adjusted so that at 750 nm the apparent absorbances of the reference suspension and of the cell suspension were identical. Apparent absorption spectra between 750 and 400 nm were recorded for several different cell concentrations. The spectra were used to calculate the in vivo Chl extinction coefficients as described in Results and Discussion.

Rates of O_2 production by *Chlorella* cell suspensions illuminated with flashing light were measured using a Clark-type electrode. The Chl concentration for all cell suspensions was 5.0 ± 0.5 μM. Saturating flashes of light at a frequency of 20 flashes/s were provided by four synchronously triggered flashlamps (Stroboslave, Type 1539A) placed symmetrically about the sample chamber. The light flashes had a measured full width of 1.6 μs and 7.5 μs at one-half and one-tenth maximum intensity, respectively.

Relative O_2 flash yields (Y_{O_2}) were measured with a Pickett-type O_2 polarograph [24]. *Chlorella* cell suspensions at Chl concentrations between 50 and 90 μM were contained in a sample chamber defined by a 0.013-cm-thick Teflon spacer with a 1.0 cm diameter circular cutout clamped between the lucite top and bottom halves of the polarograph. The floor of the sample chamber was a large area (1.3 cm^2) bare platinum electrode. A dialysis membrane formed the top of the sample chamber and separated it from an upper chamber in contact with air. No noticable desiccation of the sample occurred over the course of an experiment (15–20 min). We calculate from the average diameter of an algal cell (about 4 μm), that the settled cells covered between 25 and 45% of the floor area.

The platinum electrode was polarized to -0.7 V with respect to the reference electrode (2 g of silver wool in 0.1 M KCl) and a d.c.-coupled feedback amplifier circuit was used to operate the polarograph in a voltage-clamp mode. The output of the circuit was recorded on one channel of a Hewlett-Packard 7702A two-channel recorder equipped with an 8802A preamplifier. The pulse of O_2 produced by algae illuminated with a laser flash rose within the 7 ms response time of the recorder and decayed as a single exponential with a time constant of about 200 ms. We considered the maximum amplitude of the electrode current following a flash to be proportional to the total O_2 produced by the algae as a result of the flash.

Submicrosecond flashes of light were provided by a Phasar DL2100C flashlamp-pumped dye laser. The dye solution was 50 μM Rhodamine 6G/0.1% lauryldimethylamine oxide in methanol. The maximum laser emission was at 596 nm with a full bandwidth at half maximum of 7 nm. The laser light was focused into a randomized fiber-optics light-pipe, the exit of which was held about 2 mm above the platinum electrode surface. The laser light leaving the light-pipe was observed to uniformly illuminate the algae.

The absolute energy of each laser flash at the electrode surface was determined via a thin glass beam-splitter and an Epply thermopile. The output of the thermopile was recorded with a Hewlett-Packard 8803 d.c. preamplifier and the second channel of the recorder used for the O_2 measurements. The thermopile response was calibrated by substituting a bolometer (Scientech Model 360203 Laser Power Meter) in place of the polarograph at the exit of the light-pipe. We measured the platinum electrode surface to reflect $75 \pm 5\%$ of the laser light in both the presence and absence of algae. Therefore, total laser flash energies before attenuation by filters were calculated as 1.75-times the bolometer reading.

The laser flash energy at the electrode surface was varied with filters inserted into the light path after the beam-splitter but before the focusing lens. The filters were various concentrations of copper sulfate and were calibrated by direct measurement of transmitted laser light. At the start of an experiment, filters were selected so that laser flash energies were saturating (i.e., produced the maximum Y_{O_2}). Flash energies were decreased from saturation levels by the addition of filters. Flash energies were increased from saturation levels by substitution of filters already in place with filters having greater transmission, increasing the laser charging voltage, or both. Increasing the laser charging voltage resulted in flashes of greater maximum energy and greater duration. For laser flash energies of less than about 10^{16} photons · cm^{-2} at the electrode surface, the complete duration of the laser flashes was between 450 ns and 500 ns. At the highest flash energies, the complete laser flash length was 750 ns.

Chlorella cells on the electrode surface were illuminated with a train of saturating laser flashes at a rate of one flash every 2 s. After Y_{O_2} had become constant (steady state), the energies of two consecutive laser flashes were varied. Laser flash energies were then made to return to the preceding saturating levels and flash illumination was continued. After steady-state O_2 yield had been reestablished, laser flash energy was again changed. In this way, an entire light saturation curve could be rapidly obtained from the first variable flash presented to cells in steady-state conditions.

Fig. 1 shows representative data from one such experiment. The laser flash energy (upper trace) varies randomly by up to 20% from shot to shot. In contrast, the light-saturated steady-state O_2 flash yields (lower trace) are constant ($\pm 2\%$). The small variations in the saturated Y_{O_2} set the practical limits of our relative flash yield measurements at $\pm 3\%$. When laser flash energies were attenuated (brackets), Y_{O_2} decreased. At subsaturating flash energies, the yield of the second attenuated flash is always less than that of the first attenuated flash. This is the expected consequence of the flash frequency, the kinetics of deactivation of the O_2-producing system, and the statistical nature of light absorption by the photosynthetic apparatus. At low flash energies, some reaction centers are missed by consecutive flashes and, if the time between hits is sufficiently long, the O_2 producing system deactivates. The small oscillations in the light-saturated Y_{O_2} seen in Fig. 1 for flashes immediately following the two attenuated flashes demonstrates this dark-adaptation effect. The shape of the light saturation curve measured for the first attenuated flash is not affected by the deactivation effect, and, if measured following the establishment of the steady state, is not dependent on flash frequency.

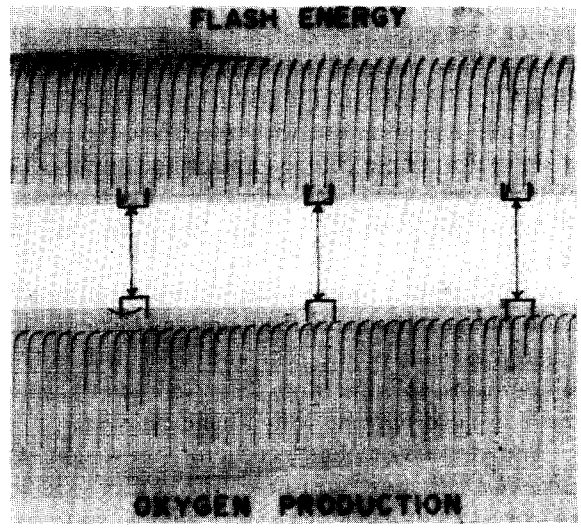

Fig. 1. Simultaneous recording of laser flash energies and relative oxygen flash yields. The upper trace records the output of the thermopile used to monitor the total energies of the laser flashes before attenuation. The lower trace shows the output of the oxygen detection circuit. At the times indicated by the brackets, the laser flashes were attenuated with filters placed in the optical path after the point monitored by the thermopile.

O_2 flash yields were normalized to the steady state: $Y_{O_2} = Y_1 / Y_s$ where Y_s is the last steady-state O_2 flash yield prior to a change in laser flash energy and Y_1 is the yield of the first flash at the new flash energy.

Results and Discussion

Optical cross-sections

We determined the effective optical cross-sections per Photosystem II trap from light saturation curves for oxygen production by *Chlorella* cells illuminated with laser flashes of variable en-

ergies (see Materials and Methods section). Fig. 2 shows the results of one such experiment. Total flash energy per cm^2 at 596 nm at the electrode surface varies by more than 5 orders of magnitude; from about 10^{12} photons/cm^2 per flash to $5 \cdot 10^{17}$ photons/cm^2 per flash. As flash energies increase from their minimum values, Y_{O_2} first increases to a maximum and then remains constant over a roughly 50-fold further increase in flash energies. At still higher flash energies, Y_{O_2} slowly declines.

The decline in Y_{O_2} at very large flash energies is completely reversible. Identical flash yields are obtained from saturating flashes given to algae before and after several hundred supersaturating flashes. Thus, Photosystem II traps have not been damaged or destroyed by the supersaturating flashes, but have, in effect, been missed. We believe this behavior is the result of a total annihilation process which occurs at high flash energies. We intend to consider this effect in more detail in a separate report [25].

For all but the greatest flash energies, the light saturation behavior of the oxygen flash yields is well described by the 'cumulative one-hit' Poisson distribution:

$$\text{Relative flash yield} = 1 - \exp(-\sigma_{O_2} E) \qquad (1)$$

where E is the energy per cm^2 of the laser flash at the electrode surface and σ_{O_2} is the effective optical cross-section for a Photosystem II trap. A

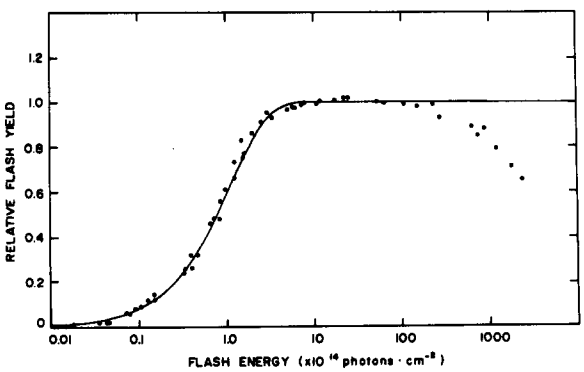

Fig. 2. Light-saturation behavior of oxygen flash yields in *Chlorella vulgaris* Relative oxygen flash yields (closed circles) are plotted vs. the total energy of the laser flashes at the electrode surface. $4.8 \cdot 10^{-16}$ mol Chl/cell; Chl b/Chl a=0.30. The solid curve is the cumulative one-hit Poisson saturation function (see Results and Discussion) calculated for $\sigma_{O_2} = 90$ Å^2.

Photosystem II trap (RCII) is defined as a site at which the energy of an absorbed photon can be used to produce the observed Photosystem II effect (in this case, oxygen evolution). The simple light saturation behavior described by Eqn. 2 will occur if all the RCII have identical optical cross-sections and if the same effect is produced by all RCII which are hit one or more times (i.e., receive the energy from one or more photons) during a flash. If the effect varies during a flash, i.e., if the quantum yield of energy transfer or primary photochemistry is less than unity, the measured cross-section will be correspondingly reduced. However, inactive RCII, or those reversing after the flash, or inefficiencies in RCI do not affect the measured optical cross-section.

The identical exponential saturation behavior will be obtained if two of more traps share a common antenna, provided there is no difference in the probability of escape from open and closed traps (vide infra). Under these conditions, when t traps share an antenna, the optical cross-section of which is σ_a, the cross-section determined from the light saturation curve will be the average optical cross-section per trap: $\sigma_{O_2} = \sigma_a / t$. The measured quantity $\sigma_{O_2} E$ is the average number of absorbed photons (hits) per trap. The number of hits on the larger 'unit' consisting of the t traps and antenna will be t-times greater. Mauzerall [20,21] has used the distinction between hits per trap and hits per unit to deduce from fluorescence yield measurements that, in *Chlorella*, about four traps share a common antenna. This larger unit may represent a distinct physical association or may represent the average distance an excited state can travel during its lifetime in the PS II antenna.

The solid curve in Fig. 2 is the cumulative one-hit Poisson distribution (Eqn. 1). The position of this theoretical curve along the flash energy axis was adjusted to give the best fit by eye to the experimental results. The value of σ_{O_2} obtained from the fit (most conveniently from the flash energy when $\sigma_{O_2} E = 1$) is a measure of the physical size of the target presented by a Photosystem II trap and its associated pigment antenna to the incoming radiation. For the *Chlorella* cells used in Fig. 2, σ_{O_2}, the average effective optical cross-section at 596 nm per RCII is about 90 Å^2. If Eqn. 1 exactly described the light saturation be-

havior of the oxygen flash yields, the sum of the errors in the measurements involved in a single experiment under the best conditions allows a determination of σ_{O_2} to about $\pm 5\%$.

Heterogeneity in PS II cross-sections

The curves in Fig. 3 allow us to set some limit on the extent of heterogeneity in RCII antenna sizes in *Chlorella*. The data of Fig. 2 have been replotted with the laser flash energies recalculated as the average number of hits per trap (x/t). Curve a is the cumulative one-hit Poisson saturation function obtained using a single average cross-section per trap. Curves b and c were calculated assuming square distributions of cross-sections where the largest cross-section (σ_{max}) was 3- or 10-times greater, respectively, than the smallest cross-section (σ_{min}). To facilitate comparison, curves b and c have been shifted along the x/t axis to match curve a at $x/t = 1$ (the average σ). It is clear that the data do not support a 10-fold distribution in cross-sections. However, a 3-fold range can be accommodated within the scatter of the data. Other calculated distributions (triangular and Gaussian) yielded curves of similar shape, although, due to the exponential weighting, the measured σ_{O_2} would also be slightly larger (10–15%)

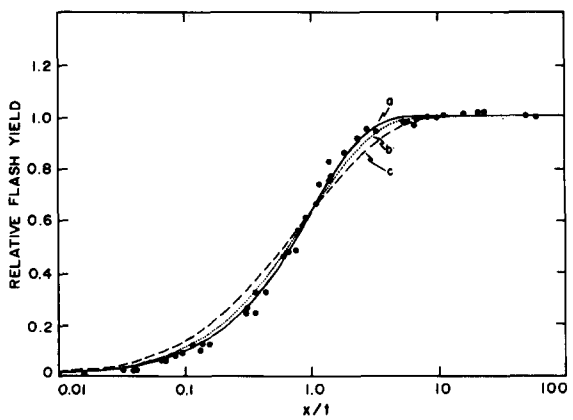

Fig. 3. Effects of heterogeneity in σ_{O_2} on the light saturation behavior of oxygen flash yields. The data (closed circles) are replotted from Fig. 2. The flash energies are calculated as hits per PS II trap (x/t). Curves a (————), b (· · · · · ·) and c (- - - - - -) were calculated assuming rectangular distributions of cross-sections and are shifted horizontally for best fit. The ratios of the largest cross-section to the smallest cross-section ($\sigma_{max}/\sigma_{min}$) were 1.0, 3.0 and 10.0 for curves a, b and c, respectively.

than the average. In a similar manner, bimodal distributions of equal amplitude but large separation ($\sigma_2 \geq 3\sigma_1$) can be excluded by the data while more moderate ranges are allowed. We conclude that if a distribution in PS II antenna sizes exists in *Chlorella*, it cannot exceed a factor of about 3.

Several recent measurements have produced evidence for some degree of heterogeneity in PS II antenna sizes within a single organism. Melis and co-workers used the kinetics of fluorescence induction [26] and the light-induced absorbance change at 320 nm [27,28] to deduce the existence of a bimodal distribution in the size of the antenna associated with RCII in chloroplasts isolated from higher plants. In contrast, Jursinic [29] used a continuous distribution in RCII antenna sizes to fit his measurements of the flash intensity-dependent variations in the sizes of Joliot-Kok oscillations in O_2 flash yields in *Chlorella*. Arntzen et al. [30,31] measured 'photosystem' sizes in spinach chloroplasts by electron microscopy. They found that the sizes of the particles located on different membrane fracture faces were smoothly distributed about uni- or bimodal averages. In general, all of these measurements agree that the range in sizes of the antenna associated with RCII does not exceed a factor of 2 to 3. It is interesting to note that, due to the statistical nature of light absorption by the photosynthetic apparatus, the light-saturation behavior presented by such moderate distributions in cross-sections is practically indistinguishable from that obtained with a single cross-section. In terms of the physiology of the organism, the two cases are effectively identical.

Another source of heterogeneity which will broaden the light-saturation curve is non-uniform illumination of the sample. This can occur if the light field itself is inhomogeneous or if the sample is optically thick (absorption plus scatter). To minimize this effect, we have used randomized fiber optics to illuminate a dilute sample (less than 'half-monolayer') of cells on the platinum electrode with 596 nm light. We find significant broadening of the light-saturation curve if we increase the cell concentration to levels corresponding to as little as 0.7–1.0 'monolayer'. The observed curve shape under these conditions is very similar to that obtained from a bimodal distribution with a minor component having a smaller σ_{O_2}.

Escape at closed and open traps

Some experiments have suggested that energy arriving at a closed PS II trap can escape the trap and move to other PS II traps [32–34]. It has also been suggested that excitation energy may escape from previously open traps, a process called 'de-trapping' [35,36]. The statistical effects of these occurrences on the flash saturation curves have been calculated [20,21]. Escape from closed traps leads to a sharpening of the saturation curve relative to the cumulative one-hit Poisson curve. The limiting case (escape probability of 1, infinite number of traps) is a completely linear relationship between flash energy and yield with a break to a curve of zero slope at the maximum yield. Conversely, escape from open traps broadens the saturation curve. The limiting case is obviously zero yield for all flash energies. Interestingly, an equal probability of escape at both open and closed traps practically reforms the exponential saturation behavior. In this case, the measured σ_{O_2} is unaffected by the escape but the measured lifetime of an excited state in the PS II antenna is lengthened. Most of our experimental curves tend to be a bit sharper than exponential (cf. Figs. 2–4) and thus suggest that the probability of escape from closed traps exceeds that for open traps. No distribution of cross-sections could ever sharpen the saturation curve.

Effect of growth irradiance on σ_{O_2}

The irradiance at which *Chlorella* cells are grown affects the measured value of σ_{O_2}. The effect is shown in Fig. 4 for cells grown in the high-light and low-light fields described in Materials and Methods. The low-light-grown cells used to obtain the data shown in Fig. 4 contain about 8-times more Chl than do their high-light grown counterparts and are relatively more enriched in Chl *b* (see Table I). From the curves shown in Fig. 4, we calculate that σ_{O_2} decreases from about 115 Å^2 in the low light grown cells to 44 Å^2 in the high-light cells. The change in σ_{O_2} with growth irradiance is a direct and unambiguous demonstration that, in addition to the commonly observed changes in total intracellular pigment content, *Chlorella vulgaris* cells adapt to growth in different environmental light levels with specific changes in the average effective optical target size per PS II trap.

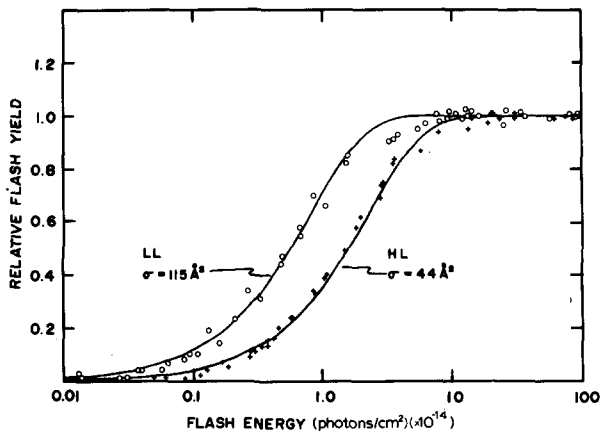

Fig. 4. Effects of growth irradiance on σ_{O_2} in *Chlorella*. Cells were grown in the low light (LL, open circles) and high light (HL, crossed) fields as described in Materials and Methods. σ_{O_2} for the saturation curves are shown. $1.7 \cdot 10^{-16}$ mol Chl/cell, high light; $13.6 \cdot 10^{-16}$ Chl/cell low light.

The measured light saturation behavior of the O_2 flash yields from low-light-grown cells deviates from the simple one-hit Poisson saturation curve as laser flash energies approach saturating levels (Fig. 4). This behavior may be the result of a wide distribution of RCII antenna sizes within these cells but it is particularly suggestive of a minor subpopulation with a much smaller cross-section. This behavior could also result from an inhomogeneous distribution of light through the cells due to their heavy pigmentation. Inhomogeneous absorption within a single cell would decrease the in vivo absorption cross-section for chlorophyll (sieve effect). However, we find (vide infra) no difference in the 596 nm in vivo absorption cross-sections of chlorophyll in high- and low-light growing cells. Thus, in addition to the large average optical cross-section per RCII low-light-grown cells apparently have a wide or bimodal distribution of RCII. For the remainder of this report we will use the values of σ_{O_2} for these cells taken from the simple saturation curves as shown in Fig. 4.

Conversion of σ_{O_2} to RCII antenna size.

To convert our measured values of σ_{O_2} into the equivalent number of pigment molecules acting as antenna for RCII we must determine the in vivo absorption cross-section of the pigments. We mea-

TABLE I

IMPORTANT PHOTOCHEMICAL PARAMETERS OF *CHLORELLA VULGARIS* CELLS GROWN AT DIFFERENT IRRADIANCE LEVELS

Irradiance [a]	Chl/cell [b]	Chl b/Chl a	$\sigma_{O_2}(Å^2)$	Chl $(a+b)$/RCII	PSU_{O_2} [c]	$1/\phi_{O_2}$
Low	15.7	0.36	110	380	5460	14.4 *
Low	13.6	0.34	115	400	4500	11.2
Intermediate	5.00	0.30	75	260	2250	8.7
Intermediate	3.73	0.25	57	200	1700	8.5
Intermediate	3.33	0.24	60	210	2200	10.5
Intermediate	3.08	0.26	75	260	2380	9.2
High	1.66	0.15	44	150	1540	10.3
High	1.62	0.15	38	130	1360	9.7

[a] Light levels in erg.·cm^{-2}·s^{-1}: High, $(2-3)\cdot 10^5$; intermediate, $8\cdot 10^3$; low, $(1-2)\cdot 10^2$.
[b] $\times 10^{-16}$ mol Chl $(a+b)$/cell.
[c] mol Chl $(a+b)$/mol O_2/flash.
[d] Photons absorbed/O_2 produced.

sured apparent whole cell absorption spectra for suspensions of *Chlorella* cells as described in Materials and Methods. Fig. 5 shows plots of the apparent absorbance at selected wavelengths by cell suspensions vs. concentrations of the suspensions. For all visible wavelengths, the apparent absorbance of the cell suspension was a linear function of cell concentration. However, the y-axis intercept was not 0 in most cases. The residual apparent absorbance, which depended on both wavelength and cell absorbance, represents an uncorrected scattering artifact.

We calculated whole cell 'extinction efficients' from the slopes of the absorbance vs. cell concentration plots. Fig. 6 shows a plot of the absorption spectrum calculated for a 10^7 cell/ml suspension of *Chlorella* cells in a 1.0 cm path cuvette. From the chlorophyll content of these cells ($4.3\cdot 10^{-16}$ mol Chl $(a+b)$/cell) we can determine the in vivo cross-section of a chlorophyll molecule at

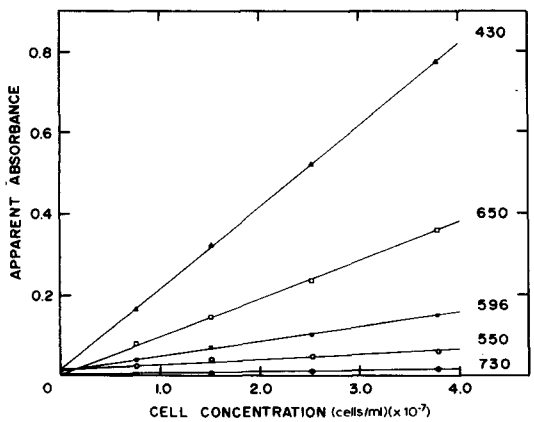

Fig. 5. Apparent absorption vs. cell concentration. The apparent absorption of a suspension of *Chlorella* cells in a 1 cm pathlength cuvette is shown plotted as a function of cell concentration for the five wavelengths: 730, 650, 596, 550 and 430 nm. Apparent absorption spectra were measured as described in Materials and Methods.

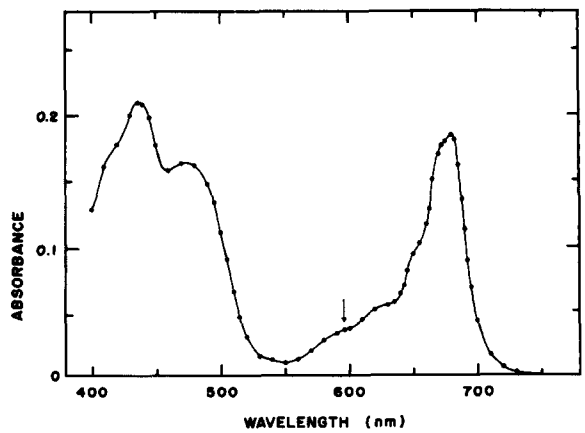

Fig. 6. Calculated absorption spectrum of a 10^7 cell/ml suspension of *Chlorella* cells. The calculation was made assuming a 1 cm pathlength. The arrow indicates the laser wavelength. $4.3\cdot 10^{-16}$ mol Chl/cell; Chl b/Chl a=0.26.

any wavelength where chlorophyll *a* and chlorophyll *b* are the only absorbing pigments (wavelengths greater than about 530 nm). At laser wavelength (596 nm, indicated in Fig. 6 by the arrow), the in vivo cross-section of chlorophyll (σ_{Chl}) was determined from several such measurements to be 0.29 ± 0.01 Å^2. For a suspension of randomly oriented molecules such as chlorophyll in the chloroplast membranes of *Chlorella* the relation between optical cross-section and decadic molar extinction is σ_{O_2} (Å^2/molecule) $= 3.82 \cdot 10^{-5}\ \epsilon$ ($M^{-1} \cdot cm^{-1}$).

We found no significant differences in σ_{Chl} measured on low-light-grown or high-light-grown cells. These cells differed by a factor of about 2 (Table I) in their relative Chl *b* contents. Thus, it seems that at 596 nm the in vivo cross-sections of Chl *a* and Chl *b* are similar and a single value for σ_{Chl} can be used to calculate antenna sizes of RCII. The optical cross-section we determine by this method measures the true light absorption properties of chlorophyll in its biological environment and properly includes any effects of heterogeneous absorption (flattening or sieve effect) within the cell. For low cell densities, the rate of light absorption by chlorophyll in vivo is exactly given by $\sigma_{Chl}E$ for any wavelength where σ_{Chl} and E are known.

Table I contains measured values for total cellular chlorophyll content, Chl *b*/Chl *a*, and σ_{O_2} for *Chlorella* cells grown in the three types of light field described in Materials and Methods. In addition, the effective antenna size per PS II trap, calculated as $\sigma_{O_2}/\sigma_{Chl}$, is shown in each case. The total chlorophyll content is almost 10-times greater in the low-light-grown cells than in the high-light-grown cells. In contrast, σ_{O_2} only corresponds to a 3-fold range in RCII antenna sizes from 130 to 400 Chl ($a + b$)/RCII. We find that *Chlorella* cells growing in 'normal' laboratory conditions have an average RCII antenna size of 210–260 Chl ($a + b$)/RCII (Table I, intermediate irradiance). This size compares well with the commonly computed estimate of 250–300 (see Ref. 37 for a discussion of this estimate) but the similarity ends there. Unlike such estimates, our values for the RCII antenna size are derived from measured optical cross-sections for photon absorption by the RCII antenna and are thus absolute measurements. They do not depend on assumed mechanisms, reaction-center ratios or the absence of inactive reaction centers.

The difference in the total chlorophyll content between cells growing in the high- and low-light fields is more than 3-times greater than the concomitant change in RCII antenna sizes. This result may reflect an increase in the average RCII content per cell in low-light-grown cells relative to high-light-grown cells. It would also be obtained if a significant fraction of the chlorophyll in both types of cells (e.g., that in the antenna of RCI) functions poorly as antenna for RCII. Our measurements (vide infra) show that the minimum quantum requirement for photosynthesis in flashing light remains constant at about 10 for the cells grown in all three light fields. We conclude that both mechanisms occur. A 2–2.5-fold difference results from the distribution of chlorophyll as effective antenna for RCII and RCI and the remaining difference arises from an increase in cellular reaction center content. A quantative analysis is in preparation (Ley, unpublished data).

Minimum quantum requirement for photosynthesis in flashing light

In addition to σ_{O_2}, the light saturation curve for O_2 flash yields contains information on the quantum requirement of O_2 production in photosynthesis in flashing light. Under optically thin conditions, the total light absorbed by a suspension of cells containing n Chl molecules and illuminated with a short flash of total energy E is: $n \cdot \sigma_{Chl} \cdot E$. In the steady state, the O_2 yield produced by the flash is $n \cdot Y_c \cdot (1 - \exp(-\sigma_{O_2}E))$. Y_c is the maximum obtainable O_2 flash yield per Chl molecule (the inverse of the photosynthetic unit size for O_2 production, PSU_{O_2}, also called the 'Emerson and Arnold number') and σ_{O_2} is the measured optical cross-section for O_2 production. For flash energy E the quantum requirement (QR) for O_2 production is:

$$QR = \frac{n \cdot \sigma_{Chl} \cdot E}{n \cdot Y_c \cdot (1 - \exp(-\sigma_{O_2}E))} \qquad (2)$$

The minimum quantum requirement for O_2 production in flashing light (which is the inverse of the maximum quantum yield, ϕ_{O_2}) occurs when E is small and $1 - \exp(-\sigma_{O_2}E) = \sigma_{O_2} \cdot E$:

$$1/\phi_{O_2} = \frac{(PSU_{O_2}) \cdot \sigma_{Chl}}{\sigma_{O_2}} \qquad (3)$$

Eqn. 3 summarizes the relationship between parameters which have been the subjects of nearly 50 years of research on the light requirements of photosynthetic O_2 evolution.

The oxygen polarograph, although having great sensitivity and rapid response characteristics, cannot be used for the quantitative determinations of O_2 production. We measured PSU_{O_2} values using a Clark-type electrode to determine the rate of O_2 production by algae illuminated with a series of saturating flashes from flashlamps (described in Materials and Methods). We used the oxygen polarograph to check that the relative yield of a saturating flash from the flashlamps and from the laser were identical. This point is crucial since it shows that under our conditions, maximum oxygen flash yields are independent of both the length (0.3 vs. 3 μs) and the color (597 nm vs. 'white') of the flash.

PSU_{O_2} values measured for the algae grown in the various light fields are included in Table I. Similar to total cell chlorophyll content, Chl *b*/Chl *a*, and σ_{O_2}, PSU_{O_2} values increased as cells were grown at lower light levels. Measured values for PSU_{O_2} increased roughly 4-fold from about 1300 to about 5500 Chl $(a+b)/O_2$ over the range of light levels used to grow the cells.

The final column in Table I contains calculated values for the minimum quantum requirement for photosynthesis in flashing light, $1/\phi_{O_2}$, by cells grown in the various light fields. Unlike any of the other measured quantities, $1/\phi_{O_2}$ is essentially constant at all light levels (with the possible exception of the cells containing the most chlorophyll, starred). If the starred value is excluded, $1/\phi_{O_2}$ calculated from all other measurements is 9.7 ± 1.0 (mean $\pm$ S.D.). If the starred value is included, $1/\phi_{O_2}$ is 10.3 ± 1.9. We conclude that the minimum quantum requirement for O_2 production by *Chlorella* cells in flashing light is independent both of total intracellular pigment content and of the antenna size of PS II and is constant at 10 ± 1 photons absorbed per O_2 evolved.

Since $1/\phi_{O_2}$ is practically invariant despite a 3-fold change in the average PS II antenna size, it seems likely that both the efficiency with which light absorbed by the PS II antenna is delivered to RCII and the efficiency of RCII photochemistry are independent of PS II antenna size. Furthermore, $1/\phi_{O_2}$ is also essentially unaffected by a 10-fold change in total cellular chlorophyll content. This observation suggests that the fraction of the total cell chlorophyll contained in the summed RCII antennae does not vary greatly over this range.

Our measured value for $1/\phi_{O_2}$ in flashing light of 10 ± 1 is essentially identical to that measured for *Chlorella* and other microalgae using dim continuous light [38,39]. This is the first direct demonstration of the often assumed equality of the two measurements. One important consequence of this observation is that the PSU_{O_2} can be converted to the average PS II antenna size using the measured $1/\phi_{O_2}$ of 10 (not 8). We note that under optimal conditions, *Chlorella* cells show a minimum quantum requirement for growth which is very close, if not identical to the measured values for the minimum quantum requirement for O_2 production [40,41].

The measured $1/\phi_{O_2}$, 10, is 25% greater than the theoretical minimum of 8 imposed by a literal interpretation of the 'Z-scheme' (i.e., identical numbers of RCI and RCII acting in series with quantum yields of unity for linear electron transport). Such an increase can arise from two different sources: (1) inefficient light harvesting or photochemisty by RCI or RCII; or (2) an imbalance in the absorption properties of the RCI and RCII antenna.

Two broad categories of inefficiencies in photosynthetic photochemistry can be distinguished by their effect on O_2 production in flashing light. Reaction centers which are closed or inactive for the duration of the flash or which undergo a back-reaction after the flash do not contribute to O_2 production by the flash. This type of inefficiency reduces the maximum attainable flash yield and thus increases the measured PSU_{O_2}. The measured σ_{O_2}, however, is not affected and is identical to the in vivo absolute absorption cross-section per RCII. In contrast, inefficiency in energy transfer from antenna to trap or quantum yields of less than unity for photochemistry during the flash by open, active traps will result in a measured σ_{O_2} smaller than the actual in vivo absorption cross-section per RCII. PSU_{O_2} will be maximum, corresponding to one electron transferred per RCII per saturating flash. In either case,

$1/\phi_{O_2}$ calculated from the ratio of σ_{O_2} and PSU_{O_2} will exceed the theoretical minimum. Sun and Sauer [42] and Avron and Ben-Hayyim [43] have shown that both RCI and RCII can use light absorbed by antenna pigments for the reduction of artificial acceptors with quantum yields close to unity. No experimental distinction has been made between RCI photochemistry involved in cyclic and non-cyclic electron transport. If RCI functions as the sole oxidant of the reduced products or RCII, cyclic electron transport may result in an increase in PSU_{O_2} and thus in $1/\phi_{O_2}$.

If reaction center photochemistry functions with perfect efficiency, the increase in $1/\phi_{O_2}$ above the theoretical minimum implies that at 596 nm in whole cells the sum of the absorption cross-sections for all RCI and their antennae is 25% greater than that for RCII. If the in vivo absorption cross-sections for antenna Chl serving RCI and RCII are identical, the summed RCI antenna will be numerically larger as well. Finally, we point out that, depending on the ratio of RCI to RCII in a cell, the absorption cross-section per RCI may be greater than, equal to, or less than that of RCII. Experiments are in progress to measure directly the absorption cross-sections for RCI and thus to distinguish clearly these possibilities.

Acknowledgement

This research was supported by the U.S. Department of Agriculture under grant number 5901941 9-03289 and the National Science Foundation grant number PCM 80114.

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