Use of a Light-Induced Respiratory Transient to Measure the Optical Cross Section of Photosystem I in Chlorella

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ABSTRACT

A method has been developed whereby the magnitude of a transient in O₂ uptake attributable to photosystem (PS) I activity, following single-turnover laser flashes of varying energy, can be used to measure the optical cross section of PSI. As measurements are made under the identical physiological conditions for which the cross section of PSII has previously been determined (AC Ley, DC Mauzerall 1982 Biochim Biophys Acta 680: 96-105), it is now possible to simultaneously measure the cross section of both photosystems in intact, photosynthetically competent cells, without the use of inhibitors or artificial mediators of electron transport. Plots of light-saturation behavior of the respiratory oscillation following pulses at 596 nanometers indicate a mean optical cross section similar to that of PSI at this wavelength, but suggest significant heterogeneity in the cross section of PSI. If this method measures only PSI activity, this result implies that there exist units with different numbers of identical chromophores, or units having populations of chromophores with different absorption spectra.

As a means of maintaining the efficiency of photosynthesis, the optimal size of a photosynthetic unit, defined as the number of pigment molecules capable of transferring energy to a given reaction center, can vary with changing environmental conditions. It is known that several hundred Chl molecules are associated with reaction centers (RC) for PSI and PSII in plants and algae, together with many additional components such as Cyt and quinones. Recent research has made it increasingly clear that no simple or single stoichiometry of these components prevails, and that the relationships among the components of the photosynthetic apparatus can change on both a long and short time scale (1, 4, 9). Thus, an objective measure of the absolute size of the photosynthetic unit and of the distribution of the pigments between RC and PSI is a useful tool in the study of the structure and function of these systems.

The absorption properties of a reaction center and its associated pigments can be quantitatively described in terms of its optical cross section σ, the area for photon capture by the light-absorbing pigments. This quantity, which for an isotropic system varies directly with the absorbanncy index, can be translated into the number of pigment molecules functionally contributing to a particular photoreaction by use of the measured in vivo absorption cross section for a pigment molecule at the specified wavelength. At wavelengths where the different Chl have similar absorbance, the conversion to obtain the total number of Chl molecules per unit is straightforward. In cases where the absorption differs, however, direct information can be gained about how many of each of the different Chl are involved.

A method was developed by Ley and Mauzerall (6) by which the light-saturation behavior of O₂ formation by Chlorella cells in response to single turnover laser flashes was used to determine the cross section of PSII. Unlike PSII, however, PSI has no readily quantifiable product indicative of its activity, and measurements must be made of other light-induced signals or intermediates. For example, Weaver and Weaver (14) measured a light-induced EPR signal in spinach subchloroplast particles, and Greenbaum (2, 3) studied H₂ production by PSI of Chlorella cells under anaerobic conditions.

In this report, we describe a new method for the measurement of the optical cross section of PSI in Chlorella. As in the previous studies from this laboratory (5, 6), use of monochromatic, single-turnover flashes of light avoids the complicated kinetics induced by the distribution of turnover times in electron transfer reactions which determine the steady state response. Since measurements are made under exactly the same conditions as those used for the measurements of PSII, it is possible for the first time to measure the cross sections of both photosystems under identical physiological conditions without the use of artificial inhibitors or mediators of electron transport.

MATERIALS AND METHODS

Batch cultures of green alga Chlorella vulgaris were grown at 20°C in Burr's medium on a rotary shaker with continuous lateral illumination (8 × 10⁻² erg.cm⁻².s⁻¹) from cool-white fluorescent lamps. Cells (5-6 d old) were collected from exponentially growing cultures (doubling approximately every 24 h) by centrifugation at 10,000g, and were resuspended in the same medium + 1 mm NaHCO₃. These growth conditions are similar to those used by Ley and Mauzerall (6).

Relative O₂ flash yields (PSI activity) and respiratory oscillations (PSI activity) were measured with a Pickett-type O₂ polarograph as described previously (6). From the average diameter of an algal cell (approximately 4 μm), it was calculated that the settled cells covered about 35% of the area of the bare platinum electrode. The circulating buffer, the same medium as that in which the cells were suspended, was partially de-gassed by vacuum to avoid bubbles and excess contribution of oxygen to baseline variations.

Microsecond flashes of light were provided by a Candela LFDL 2 flashlamp-pumped dye laser, using a dye solution of 100 μm rhodamine 6G/0.1% lauryldimethylamine oxide in 50% methanol (maximum emission at 596 nm). The measured laser flash energy reaching the entrance to the fiber optics was decreased by insertion of calibrated filters of various concentrations of Cr, Ni, Co, and CuSO₄ contained in closed glass cuvettes (5 cm × 2.5 cm light path).

1 Abbreviations: RC, reaction center; σ, optical cross section; EPR, electron paramagnetic resonance.
Light-saturation curves for the production of O$_2$ were measured essentially according to the procedure of Ley and Mauzerall (6). Briefly, cells on the electrode surface were illuminated with a train of saturating laser flashes at the rate of one flash every second. When the amplitude of O$_2$ production had become constant, the flash energy illuminating the cells was diminished with one or more of the filters. The mean amplitude of the O$_2$ produced as a result of the first attenuated flash was recorded, after which the filter(s) was removed and flash energy returned to saturating values. In this way, an entire light saturation curve could be rapidly obtained for cells in a steady state of pulsed light.

For the measurement of the light-induced signal used here as an indication of PSI activity, algae were first dark-adapted by covering the electrode system with several layers of black cloth in a very dimly lit room for 3 min (such that no O$_2$ evolution could be measured following the first two flashes of saturating laser light delivered to the cells). Cells were then illuminated by the specified number of laser flashes of the indicated energies before returning to dark conditions. For measurement of the amplitude of respiratory oscillations following illumination, the difference between the maximal (approximately 20 s after the laser flashes) and minimal values (approximately 30 s later), corresponding to the $T_1$ and $T_2$ of Ried (10, 11), from the linear baseline was recorded as the amplitude of the oscillation.

O$_2$ flash yields and amplitudes of respiratory transients were normalized to control values (measurements made at saturating energies) repeated at frequent intervals during each experiment, and data plotted as the relative amplitudes.

RESULTS AND DISCUSSION

For the measurement of PSI activity, we have made use of a transient in the rate of O$_2$ uptake exhibited by Chlorella cells following one or more single-turnover flashes. This response appears as a net decrease in uptake approximately 30 s, followed by an overshoot in the opposite direction of similar duration, and a series of damped oscillations before returning to baseline levels of respiration (Fig. 1).

Ried (10, 11) found this behavior to be independent of oxygen evolution in Chlorella and Scenedesmus because it was not inhibited by DCMU, an inhibitor of photosynthetic oxygen production. On the other hand, this oscillatory activity was inhibited by 5 mm glucose, which did not affect O$_2$ production. Furthermore, the action spectrum of the magnitude of the oscillations followed that attributable to PSI activity (12, 13). The oscillations occur only in the presence of CO$_2$ or HCO$_3^-$, and are best observed in dark-adapted cells (they do not occur in cells exposed to white background illumination greater than 6 x 10$^{-2}$ mW/cm$^2$; data not shown). The magnitude of the oscillations following saturating laser flashes is not noticeably diminished by the inclusion of 10 $\mu$m DCMU in the circulating buffer (NL Greenbaum, D Mauzerall, unpublished data), thereby rendering unlikely any contribution by electron transport from PSII.

The first amplitude of the oscillations is linearly dependent upon the number of flashes of saturating or of subsaturating energy (Fig. 2). It is observed that a response of proportional magnitude is elicited by a single laser flash, and that there is no variation associated with S-state periodicity. Thus, the oscillation, whose basic cause and function is as yet uncertain, has the desirable properties of a linear measure of photoexcitation of PSI.

The saturation behavior of a light-induced response, under certain well-defined conditions, is described by the cumulative single-hit Poisson distribution:

Relative yield = $1 - \exp(-\alpha E)$

where $E$ is the energy per cm$^2$ of the laser flash at the electrode surface. The conditions necessary for this behavior are: (a) uniform illumination upon isotropic units with the same cross section in optically thin samples; and (b) the same effect is produced by all reaction centers which are hit one or more times during each single-turnover flash. The approximation of an isotropic system is acceptable in this case because of the depolarizing effect of Chl antenna systems. A saturation curve rising more steeply than a simple exponential curve is caused by an increased probability of escape of energy from closed RC with respect to open RC as the traps become filled, whereas an equal probability of escape from open and closed traps results in a curve almost identical to the simple Poissonian. A saturation curve rising less steeply than the complement of an exponential is more ambiguous. It may be caused by an increased probability of escape from open traps over escape from closed traps, by nonhomogeneous illumination, or by a distribution of cross section sizes (see Refs. 7 and 8 for further interpretation of these curves).

Figure 3A shows the results of an experiment for measurement of the optical cross section of PSII at 596 nm. Flash energies
Fig. 2. Relationship between the number of single turnover flashes and amplitude of respiratory oscillations. The magnitude of oscillations (Fig. 1) is linearly dependent upon the number of laser flashes (from 1 to at least 20) of saturating ($6 \times 10^{14}$ quanta/cm$^2$), represented by the symbol (•) or of subsaturating ($3 \times 10^{13}$ quanta/cm$^2$, ■, or $2 \times 10^{13}$ quanta/cm$^2$, ▲) energy.

Fig. 3. Light-saturation curves of A) O$_2$ flash yields and B) PSI respiratory transients in *C. vulgaris* at 596 nm. The normalized O$_2$ responses were plotted versus the total light energy per flash at the electrode surface. The solid curve fit to the flash yield data (closed circles in A) is the cumulative single-hit saturation function calculated for a unique mean $\sigma = 100$ Å$^2$. The solid curve fitted to the O$_2$ transient data attributable to PSI activity (open circles in B) is that calculated for a ten-fold heterogeneity in the size of the PSI cross section, with a mean cross section similar to that of PSII. For comparison, the curve calculated for a unique $\sigma$ has been included (dashed line).

were varied between $10^{16}$ quanta/cm$^2$ per flash (unattenuated) and $10^{12}$ quanta/cm$^2$ per flash. The solid curve is the cumulative one-hit Poisson distribution calculated for $\sigma = 100$ Å$^2$. It can be seen that, similar to the data of Ley and Mauzerall (6), the measurement of the light-saturation behavior for the O$_2$ flash yields (closed circles) has a close fit to this function, indicating a unique (i.e. within a 3-fold range for a square distribution) mean size and little or no escape from closed traps (or a probability similar to that for escape from open traps).

The light saturation curve of the respiratory oscillations (Fig. 3B, open circles) indicates a mean cross section similar to that of PSII, but shows marked broadening compared with the ex-
ponential behavior depicted in Figure 3A. While such a curve is generally indicative of nonuniformity in \( \sigma \), other sources of nonuniformity (e.g., illumination, optical thickness and anisotropy of sample, etc.) must be excluded. In these experiments, the light distribution was uniform by inspection and the objective criteria of the fit of the data of the \( \text{O}_2 \) yields, leading to the conclusion that heterogeneity in the system is limited to the optical cross section of the unit. This conclusion is also consistent with findings of Greenbaum from measurements of \( \text{H}_2 \) production by PSI in Chlorella (2, 3). Replotting of the data published by Weaver and Weaver (14), in which an EPR signal was used to measure photoexcitation of PSI of spinach subchloroplast particles at 694 nm, on a logarithmic scale yields a curve for the light saturation which demonstrates a similar deviation from the exponential curve as the data in Figure 3B.

Two possible origins of heterogeneity in \( \sigma \) are: (a) units having different numbers of identical chromophores, resulting in a distribution of cross sections, and (b) units having chromophores with different absorption spectra, resulting in discrete \( \sigma \) values. By comparing the data with the curves predicted by the respective models, the likelihood of either possibility can be evaluated. As a possible fit, the data in Figure 3B are presented against the curve predicted for a 10-fold square distribution in \( \sigma \) (an example of the first case). The second case, for which the saturation behavior would be expressed as the weighted sum of two individual Poissonian curves, cannot be adequately distinguished by the current data at 596 nm, as the Chl may well absorb similarly at this wavelength. If the second case prevails, measurement of the light-saturation behavior at other wavelengths at which the contributing chromophores have widely different absorption characteristics (e.g. in the far red) (12, 13) may permit dissection of the broad curve into its component Poissonian curves. Current experiments suggest the latter possibility is more correct.

**Note Added in Proof.** In recent experiments at 723 nm, the \( \text{O}_2 \) cross section has been measured to be 2 \( \text{Å}^2 \) and the light-saturation behavior of the respiratory transient is fit by a curve which is the sum of two Poissonians with approximately equal contributions of cross sections of 2 \( \text{Å}^2 \) and 20 \( \text{Å}^2 \) (NL Greenbaum and D Mauzerall 1987 in: J Biggins, ed, Progress in Photosynthesis Research Vol. II, Martinus Nijhoff Publishers, Dordrecht, pp 65–68). Addition of DCMU results in a single Poissonian with a \( \sigma \) of 20 \( \text{Å}^2 \), suggesting that the smaller \( \sigma \) is due to PSI. However, the amplitude of the oscillation following saturating flashes is undiminished by the inhibitor, i.e. is not decreased by half as would be expected by the above explanation. Thus, assuming DCMU itself is not the cause of the discrepancy, either PSI is heterogeneous or the respiratory effect measures a specific interaction between the two photosystems.

**LITERATURE CITED**


5. LEY AC 1984 Effective absorption cross-sections in Porphyridium cruentum. Implications for energy transfer between phycobilisomes and photosystem II reaction centers. Plant Physiol 74: 451–454


