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Photosynthetic Unit Size during the Synchronous Life Cycle of *Scenedesmus*[1]

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ABSTRACT

Apparent size of the photosynthetic unit (chlorophyll/O_2 per flash) was estimated by O_2 yield of repetitive short flashes on cell samples taken at various times from a synchronized culture (14-hour light, 10-hour dark) of *Scenedesmus obliquus*. Unit size was essentially invariant (< 10% variation) with a mean value of 1750 chlorophyll/O_2 per flash. In contrast, the light-saturated photosynthetic rate per unit chlorophyll, or turnover rate of the photosynthetic unit, varied with the life cycle, rising 40% in the first three hours of the light period and decaying slowly thereafter. The results are taken as evidence that the metabolic machinery is subject to far greater control and adjustment than is the photochemical machinery.

For steady state cultures of *Chlorella*, we reported a relatively constant apparent size of the photosynthetic unit in spite of large variation in Chl content of the cells (4). In steady state cultures there is a necessary heterogeneity in cell size. Hence an attendant question is whether or not unit size remains constant during the life cycle of a cell, especially during its period of Chl synthesis. In attempts to answer this question in synchronized cultures of *Chlorella pyrenoidosa* (Emerson strain) we were foiled by difficulties in achieving adequate synchrony. Thereupon we turned to *Scenedesmus obliquus* strain D3 on which a number of studies of synchronized cultures have been reported (5, 6, 7).

We continue to use the term photosynthetic unit because of its established meaning. We should like to know about the arrangement of light-harvesting Chl for both the system 1 and system 2 reaction centers. Actually, we can estimate the number of system 2 reaction centers in terms of O_2 yield per flash and the total Chl. Hence the photosynthetic unit retains its original operational meaning: Chl/O_2 per flash. Presumably unit size in terms of Chl/electron per flash is one-fourth as large.

MATERIALS AND METHODS

The alga used was *Scenedesmus obliquus* strain D3, originally obtained from Hans Gaffron. It was grown at 25.5 C in a 6-mm thick layer of a continuous culture device and under illumination conditions which had previously given a specific growth rate of 2.3 day^{-1} for *Chlorella pyrenoidosa* under continuous illumination (4). Cell concentration was maintained at about 1 μl of cells/ml. Input medium contained (mg/l): 810, KNO_3; 500, NaCl; 117, Na_2HPO_4; 286, NaH_2PO_4; 10, $CaCl_2$; 122, $MgSO_4$; 18, ferric citrate; 16, sodium citrate; 0.5, B; 0.5, Mn; 0.05, Zn; 0.02, Cu; and 0.01, Mo.

Cell synchrony was obtained by a 14 hr light-10 hr dark cycle. In the data to be presented, 0 and 24 hr represent the time of the dark to light transition. Most cell divisions occurred between 16 and 22 hr giving reduction of cell size from average values of 350 to 50 μm^3, (estimated from packed cell volume and cell counts). These characteristics approximate those obtained by Senger (5), though our multiplication factor is lower (our 7 × *versus* his 10 ×), and are less stringent tests of homogeneity than those applied by Berkova *et al.* (1). Because of random technical difficulties, not all desired measurements were obtained for all samples; for reasons to be noted, two different series of measurements are put together in the data presented.

Other methods for cellular characteristics and O_2 exchange under illumination by repetitive flashes or 620-nm continuous light were as previously described (4). In practice we made two measurements of O_2 exchange on separate aliquots of each cell sample: (*a*) in about 4 min of flashing light at 20 flashes/sec plus 3 min of a following dark period; (*b*) in about 4 min of continuous saturating 620-nm light at 30 mw/cm^2 plus 3 min of a following dark period. Rates given for O_2 evolution were corrected by the following dark uptake rate. The flash energy was sufficient to provide about 4 quanta/unit by the test that attenuation to 25% flash energy decreased flash yields to about 65% of maximum.

RESULTS

Chlorophyll Content. Figure 1A shows that, after an initial lag, Chl synthesis is more rapid than increase in cell volume from about 3 to 7 hr. A first series of measurements was found to have a consistent error in Chl determinations arising from error in the recorder slide-wire of our spectrophotometer. All values for the first series were corrected to those of a second series by factor 0.87. (This same normalization is used also for Fig. 1B.)

Photosynthetic Unit size. Figure 1B is addressed to the central question asked. The average value for all data is 1750 Chl/O_2 per flash ± 130 SD. There are no significant differences for values chosen for any 3-hr period within the cycle. Any variations in apparent unit size during the life cycle are less than 10%.

Repetitive *versus* Single Flash Yields. Individual flash yields observed with the more sensitive rate-measuring O_2 electrode show that repetitive flashes at 3 to 5/sec give lower yields than single flashes on a 700- or 710-nm background. This difference is understandable since repetitive flashes, which provide all

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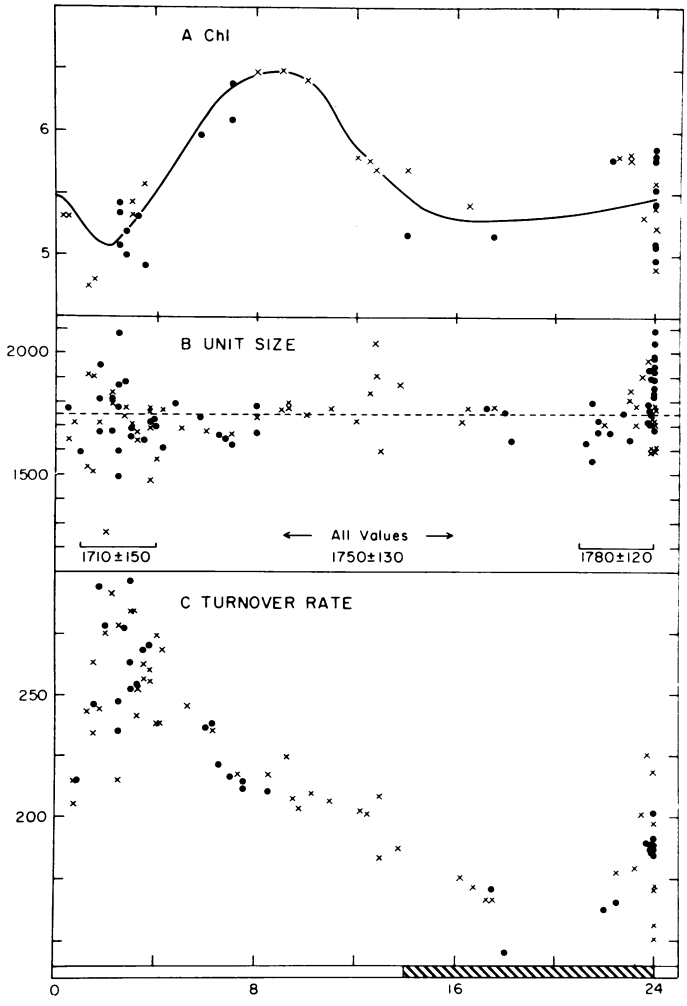

FIG. 1. Characteristics of synchronized cultures *versus* time in the cycle for a light period 0 to 14 and a dark period 14 to 24 hr. There are two series of measurements (see text): Series I (xxx) and Series II (● ● ●). A: Chl content, μmoles Chl/ml cells. Curve drawn by inspection. B: Unit size, Chl/O_2 per flash. Averages for all values and for two time periods are shown with SD. C: Maximum unit turnover rate, sec^{-1}.

Table I. *Cellular and Photosynthetic Characteristics*

Culture	Cell Characteristics			Saturation Photosynthesis		Unit	
	Sampling Time[1]	Cell Size[2]	Chl (a+b)	P_{Chl}	P_v	Size	Max. rate[3]
	hr	× 10^{12} *ml*	*μmoles Chl/ml cells*	*moles O_2/mole Chl·hr*	*moles O_2/l cells·hr*	*Chl/O_2·flash*	*sec^{-1}*
14L-10D[4]	1–4		5.2	550	2.8	1710	260
	6–8		6.4	470	2.9	1680	220
	14–16	350					
	21–24	50	5.5	380	2.4	1780	180
Continuous light[4]		230	5.4	340	1.8	1650	160

[1] Time from light-on.

[2] Packed cell volume/cell number.

[3] Maximum turnover rate of a unit at light-saturation.

[4] Specific growth rates were 0.12 hr^{-1} for continuous light and 0.14 hr^{-1} (average) for the light period of 14L-10D culture.

actinic light for photochemistry, conceivably might not keep all system 2 centers oxidized and open. We had some concern that repetitive flashes might not be properly counting all centers and even greater concern that there might be differences in fraction of centers counted, for example between 21 to 24 and 1 to 4 hr cells. Hence we compared repetitive flash yields (3/sec) with single flash yields on a 700-nm background of intensity chosen to give maximum yield (8). For dark (24 hr) cells, the repetitive flash yield was 81 and 87% of maximum in two experiments. For 3-hr light cells, the repetitive flash yield was 90 and 88% of maximum. We take the results to mean that our repetitive flashes count a large fraction (> 80%) of centers and with no substantial difference in that fraction for the two diverse cell types.

Light-saturated Rate. Table I cites light-saturated rates of O_2 evolution referred to Chl (P_{Chl}) or to cell volume (P_v). Maximum values of P_v are reached at 6 to 8 hr, as also noted by Senger (5). Our absolute values, measured in original media, are higher than his, which were measured in Warburg No. 9 bicarbonate buffer. In our measurements by Clark-type O_2 electrode, cells suspended in No. 9 buffer gave 0.7 of the rate observed in original medium + 5% CO_2; we presume that this explains the discrepancy.

Our values of P_{Chl} reached remarkably high maximum values at 1 to 4 hr. Since the unit size remained essentially constant, a simpler expression of maximum rate can be made in terms of turnover rate per unit. Further, this is obtained directly from two adjacent measurements of rate of O_2 evolution, one in flashing light and one in saturating light. The only assumption is that at 20 flashes/sec units are actually working 20/sec. During the synchronous life cycle, the turnover rate goes through marked variations shown in Figure 1C. The early peak occurs at about 3 hr and the average for the 1- to 4-hr increment is 260/sec; for comparison the average for 21- to 24-hr dark cells is 180.

Continuous Light. An advantage of our continuous culture device was that we could convert readily from a synchronized to a steady state culture without appreciable change in effective illumination per cell. Characteristics of cells grown in continuous light are given in Table I. It is clear that cells selected from various stages of the synchronized culture cannot be put together in any proportion to duplicate the heterogeneous cells of a continuous light culture. Evidently the synchronized culture reflects also effects of the dark period imposed to obtain synchrony. However, of the several characteristics cited in Table I, only one is sensibly constant and independent of cell type: the unit size.

DISCUSSION

There are two salient results of our work. The first is a further confirmation of the near constancy in size of the photosynthetic unit (Fig. 1B; Table I) even under conditions of a high rate of Chl synthesis. Evidently antennae Chl and reaction centers are laid down at about equal rates, as if unit by unit. If we are correct in ascribing the measured unit size to photosystem 2, then the apparent variations in photosystem 2 activity (5–7) must have some explanations other than a simple variation in quantum absorption by pigment system 2.

A second result is seen in Figure 1C in terms of the 40% increase in turnover rate which occurred in the first two hours of light in our synchronized culture. We suppose that such an abrupt increase reflects a rapid increase in pool sizes of intermediates between the photoreactions, possibly accompanied by increased enzymic activity in the carbon cycle. It is clear that the metabolic machinery is subject to far greater control and adjustment than is the photochemical machinery.

We are aware that our conclusions are different from those reached in studies on development of the photosynthetic apparatus in mutant algae requiring light for Chl formation (2, 3). We suggest that apparent unit size and maximum turnover rate are informative parameters for such studies.

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