Short Communication

On the Ratio of Photosynthetic Reaction Centers RC2/RC1 in Chlorella

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ABSTRACT

Chlorella pyrenoidosa (Emerson strain) was grown under high and under low irradiance. Measurements of the O2 flash yield and the P700 absorption change gave estimates for the ratio of photoreaction centers RC2/RC1 at 1.2 to 1.5. A background light 1 did not increase flash yield.

We recently reported a study of O2 flash yield in two blue-green algae (9). The characteristics found were those expected for a system which has more reaction centers for RC1 than for RC2. For example, saturating flashes behaved as a light 1. Repetitive flashes gave an O2 flash yield not significantly increased by a background irradiance of 680 nm, an extreme light 1. This experience made us wonder about the flash yield characteristics in chloroplast systems where the RC2/RC1 ratio is reported to be greater than 1 (1, 5, 6).

From this background, we were led to inquire about the reaction center ratio in Chlorella. Our inquiry is incomplete and will call attention to a problem rather than provide an answer. We have used conventional methods, the O2 flash yield for RC2 and the absorption change of P700 for RC1.

MATERIALS AND METHODS

We grew Chlorella pyrenoidosa (Emerson strain) in continuous culture under tungsten illumination as previously described (8). Our measurement of O2 exchange also has been described (8, 9). Flashing light through a Tiffen No. 8 yellow filter (transmission 0.10 at 475 nm, 0.56 at 500 nm) was provided by a FX-76 (E. G. & G.) flash tube discharged at 6, 10, or 20 Hz from a 1 μF capacitor charged to 1400 V. The intensity-time contour of the flash gave 2.0 μs to one-third and 5.1 μs to one-tenth peak intensity; of the area under the contour (total energy), 0.75 was reached at 4.4 μs and 0.95 at 16 μs. The routine test for flash saturation was that attenuation by a 25% screen did not reduce flash yield to less than 0.63, i.e., 1 - 1/e; hence, each flash provided at least 4 quanta/RC2. We estimated the concentration of RC2 as one-fourth of the O2 Chl-1 flash-1.

Estimations of P700 were made with a dual wavelength spectrophotometer, designed by B. Kok and made by G. Johnson, operated at 1.7-nm half-band width. We measured change in A of a whole cell suspension at 700 versus 735 nm caused by 3 mw/cm2 of 443-nm actinic light (65 half-band widths) in the presence of 2 μm DCMU and used an absorption coefficient 64 mm-1 cm-1 (4).

The following are details of the spectrophotometry. Beams from two monochromators, one set at 700 and one at 735 nm, were alternated by a mirrored sector at 120 Hz and manually balanced by a wedge to give a null 120 Hz signal from the photomultiplier. The alternated beams passed through a 10-mm length of the sample cuvette, a 4 × 15 mm exit diaphragm, and a Schott RG8 filter to the photomultiplier. Actinic light from a direct current source via an interference filter illuminated the entire 1.0-ml sample at 5-mm path length; it was controlled by an electrical shutter timed to give alternated light and dark periods (as 15 s). The ΔA was estimated from the light-minus-dark signals (120 Hz) observed at 1 × 10-5 A full scale and averaged over 10 light-dark cycles.

We made the following checks upon the spectrophotometry. Maximum ΔA was observed at wavelengths 700 and 701 nm. ΔA was not affected by addition of 10 μm methyl viologen. Actinic light was saturating: attenuation to 0.30 decreased ΔA only to 0.88 of maximum; at full intensity about 0.5 of maximum ΔA was obtained without DCMU. A 50% attenuation of measuring light was without effect. Addition of actinic light and resulting fluorescence from the sample raised the total photomultiplier current by about 3. This change, plus an artifact from the actinic shutter, introduced a small perturbation in amplifier read-out of ΔA from the 120-Hz difference signals for 700/735 nm. Hence, for each sample, we also took a second measurement with the monochromator wavelengths reversed (735/700 nm); the difference was about 10% and we took an average.

RESULTS AND DISCUSSION

Table I reports our measurements made upon cells grown at two different irradiances. These correspond approximately, but not exactly, to two cell types of a previous report (8), Chl was extracted and measured as previously described (8). The Chl and P700 measurements were made immediately following harvest of cell suspension from the continuous culture chamber. However, flash yield measurements were continued over the following 5 h in order to scan several conditions. (a) In the first six experiments, measurements (with and without background) were made at flash rates of 6, 10, and 20 Hz. Flash yield was independent of flash rate, a result different than that observed in two blue-green algae (9). (b) Measurements were made on darkness (zero background) and also with backgrounds of 680 and 705 nm. Several intensities of background were chosen with the criterion that they gave rates of O2 evolution more than compensating for dark respiration but not so great that the total rate in background plus flashing light exceeded linearity. For this purpose we made light intensity plots as that of Figure 1. In all measurements, we calculated flash yield from rate of O2 evolution observed (background + flash) minus the rate observed in background alone. We found no significant

1 This report represents the last joint effort of our 33 years of collaboration.
2 Abbreviations: RC1, photoreaction 1; RC2, photoreaction 2.
effects of background light on flash yield. Inasmuch as there were no evident effects of flash rate or background, we lumped all measurements (at least six) and calculated a standard deviation for each of the first three experiments on cells grown at low irradiance.

For cells grown at high irradiance, a new problem appeared. In experiment 8-09, we were surprised by an unexpectedly low average but a wide spread of values for Chl/RC2. Inspection of records showed that flash yields were drifting upward during the 5 h of measurement. In a previous study (8), this problem had not appeared because we had made each measurement upon a sample freshly drawn from the continuous culture chamber. In the 8-10 experiment, we compared aliquots from the original harvested cell suspension versus aliquots freshly harvested before measurement. Flash yields for the original suspension drifted upward with time; flash yields on separately harvested aliquots did not drift but had a considerable random variation. The latter procedure places a heavy demand on precision of turbidostatic control of a continuous culture in which volume is increasing at almost 10%/h. Because of the latter difficulty, we elected to observe the changes in a harvested suspension with time.

Figure 2 presents data of experiment 8–19. The cell suspension was held in test tubes, aerated with 2% CO₂ in air, and provided by the weak light of a small lamp in an adjacent test tube. The illumination was less than compensating for steady state growth and chosen from experience to best maintain in most algae their characteristics at harvest. Each O₂ rate measurement was done on a separate aliquot. For present purposes, we fit the data to straight lines. For RC2, the slope of the line drawn by eye represents a 1.24× increase/300 min. The corresponding slope for RC1 is almost the same and the slope between the two Chl analyses is 1.18×/300 min. It is known that Chl pyrenoidosa, after transfer from rapid growth to darkness, continues nitrate reduction and presumed protein synthesis (7). Evidently, the continued synthesis extends to photosynthetic membrane components. Hence, it is necessary that all analyses be made on a comparable time basis after harvest. For experiment 8–19, we used concentrations of Chl, RC1, and RC2 extrapolated to zero time (harvest). For the other experiments (8–09 to 8–13) our measurements were recorded in real time. For all of them we drew best-fit curves with 1.24×/300 min slope and took the zero-time intercept for RC2. Inasmuch as in these experiments RC1 and Chl measurements had been made within the first hour after harvest, we applied no correction. The data recorded in Table I for high growth irradiance were obtained by the above procedures.

We call attention to three features in the data of Table I. (a) Cells grown at lower irradiance have somewhat larger total antennas reckoned either as Chl/RC1 or as Chl/RC2 and a higher Chl b/Chl a ratio. The differences are similar to those reported previously for Chl pyrenoidosa (8) and observed also in Dunaliella tertiolecta (1). (b) Cells grown at lower intensity have a slightly higher RC2/RC1 ratio but it is not clear that the difference is significant. (c) For all cell suspensions, the RC2/RC1 ratio is greater than 1.

We consider further two of our findings. The continually increasing flash yield after harvest from a rapidly growing culture is a phenomenon not previously reported. This has the appearance of a trivial experimental result. However, it is a hidden hazard for any investigation of effects of growth irradiance on pigments and reaction centers in algae. Cultures grown at high irradiance are likely to have large amounts of storage food materials and rapid cellular synthesis continuing into a following dark period. If Chl is extracted immediately and if the more cumbersome measures of reaction centers are delayed, the effect will be one of lowering the calculated Chl/RC ratio. There seems to have been an intuitive expectation that high growth irradiance should lead to a smaller 'photosynthetic unit' (Chl/RC). A continued dark synthesis, occurring only after growth at high irradiance, could easily lead to such a result. Hence, the phenomenon is especially dangerous in that it favors an expected result.

The finding of RC2/RC1 > 1 is not novel. Values of RC2/RC1 > 1.4 have been carefully documented by spectrophotometric measurements in spinach (5) and in Pisum and Atroplex (6) and

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**Table 1. Characteristics of C. pyrenoidosa Grown at Two Irradiances**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Chl (a + b)</th>
<th>Chl b/a</th>
<th>Chl/RC2</th>
<th>Chl/RC1</th>
<th>RC2/RC1</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>μ = d⁻¹</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells grown at low irradiance; 7-30</td>
<td>8.39 0.27 620 ± 30</td>
<td>780 1.3</td>
<td></td>
<td></td>
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<tr>
<td>8-03</td>
<td>8.49 0.29 530 ± 40</td>
<td>780 1.5</td>
<td></td>
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<tr>
<td>8-05</td>
<td>8.79 0.28 540 ± 20</td>
<td>750 1.4</td>
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</tr>
<tr>
<td>Average</td>
<td>8.6 0.28 560 770 1.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells grown at high irradiance; 5 μW/cm² 680 nm</td>
<td>3.9 0.19 400</td>
<td>550 1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-09</td>
<td>3.93 1.4 420</td>
<td>550 1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-10</td>
<td>4.20 1.5 440</td>
<td>610 1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-12</td>
<td>3.70 1.3 400</td>
<td>610 1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-13</td>
<td>3.82 1.2 440</td>
<td>610 1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-19</td>
<td>3.97 1.4 480</td>
<td>610 1.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>3.9 1.4 420 560 1.3</td>
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</table>

* Calculated as described in text.
* Calculated using cell densities of 0.197 g/ml for low irradiance cells and 0.256 g/ml for high irradiance cells (8).
ratios of 1.05 to 2.3 have been reported for the diatom Skeletonema costatum using an O₂ flash yield for estimation of RC2 (1). Our own measurements fall within the range RC2/RC1 = 1.2 to 1.5.

We began this study because of concern for the apparent anomaly that arises if RC2/RC1 > 1. For a saturating, single turnover flash, maximum O₂ yield requires that RC2 be completely relaxed (Q oxidized) at the beginning of the flash. Repetitive flashes allow accumulation of sufficient O₂ for measurement; however, they also provide an actinic light which may affect the poise of Q and other components of the electron transport chain. A simple expectation arises for the case RC2/RC1 > 1: each flash should inject more electrons via RC2 than are removed via RC1 until RC2 (Q) becomes partly reduced. The result should be a less-than-maximum O₂ flash yield. Hence, we carefully examined effects of added actinic light, especially a light 1 at 705 nm which tends to oxidize Q. We could not thereby increase the O₂ flash yield and conclude that the normally measured flash yield is maximal.

The anomaly which we posed for the condition RC2/RC1 > 1 reduces to the question: how can repetitive flashes give maximum flash yield? One explanation would arise if a sizeable cyclic flow of electrons occurs around RC2. We consider such an explanation unlikely simply because of the necessary constraint on throughput quantum yield. A more likely possibility, previously suggested (1), is that RC1 turns over more than once within the time of a xenon flash. A basis for this possibility is as follows. After a very brief (0.5 μs) flash the re-reduction of P700 in chloroplasts is polyphasic (2, 3). If the primary donors plastocyanin and Cyt f are largely reduced, then a large part of the reduction occurs very rapidly with a half-time estimated at 20 ± 10 μs (3). Potentially, this characteristic provides an automatic mechanism which can induce more rapid turnover of P700 when its donors, plastocyanin and Cyt f, are partly reduced. Such a condition occurs in a light 2 or under repetitive flashes when RC2/RC1 > 1.

Evidently a feature of the photosynthetic mechanism subject to rather wide variation is the number and arrangement of reaction centers. We shall look forward to a more complete description of this feature and eventual understanding of the strategy of the RC2/RC1 ratio in different photosynthetic systems.

LITERATURE CITED