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Is there significant cyclic electron flow around photoreaction 1 in cyanobacteria?

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Abstract. Evidence for a cyclic electron flow has been sought by study of the steady-state poise of P700 and rate of photoreaction 1 in three cyanobacteria. Under an actinic light 1 (440 or 680 nm) the rate of photoreaction 1 is limited by the rate of electron supply provided by photoreaction 2 and by all return electron flow from low potential donors such as ferredoxin and NAD(P)H. Plots of p, the steady-state fraction of P700 reduced, versus the reciprocal intensity, 1/I, yield linear segments of slope Ip. From considerations of a simple model the slopes and extrapolated intercepts of the linear segments provide estimates of the rate of return electron flow. Analysis shows that the total return electron flow cannot be large, by one estimate not more than three times the rate of dark respiration. This result leads to a conclusion that cyclic electron flow (and any dependent phosphorylation) is not a significant process in these cyanobacteria at ordinary light intensities.

Abbreviations: DAD – diaminodurene; PMS – phenazine methosulfate

Introduction

In a previous report [11] I described the interplay of respiratory and photosynthetic electron transport in the cyanobacterium *Agmenellum quadruplicatum*. Respiratory electron flow, which proceeds to O_2 via cytochrome oxidase in the dark, can be diverted in light to reduction of P700. By using P700 or Cytochrome (f + c533) as internal redox indicators it was possible to titrate the relatively steady respiratory reducing rate by the photoxidation rate produced with various actinic intensities of a light 1. With added DCMU, which made 440 nm light a "perfect" light 1, the titration of P700 gave an interesting result: the product of intensity (I) times fraction (p) of P700 reduced became constant. Evidently the Ip product, the relative rate of photoreaction 1, becomes a measure of the total electron flow rate to P700. All of these observations were expected from results of in vitro studies. However, one further result was not expected.

In *Ag. quadruplicatum*, as in other cyanobacteria, the dark respiratory O_2 rate is low, a few percent of the photosynthetic O_2 rate. The respiratory rate could be reduced ($\times 0.3$) by dark starvation and then increased ($\times 2$) by addition of the substrate glycerol. These changes in respiratory electron flow, though small compared to potential photosynthetic electron flow, made significant changes in P700 and in the Ip product in the presence of DCMU. The experimental result led to two unexpected conclusions: (1) any cyclic electron flow around P700 cannot be large compared to the electron flow supported by dark respiration; (2) cyclic phosphorylation cannot make significant contribution to the photosynthesis of this cyanobacterium growing at ordinary light intensities.

I was tentative in presenting the above conclusions and invited shortcomings perceived in the argument [11]. One important criticism quickly became apparent. In reconstituted chloroplast systems cyclic phosphorylation is known to depend upon proper poise of the added carrier (as phenazine methosulfate or ferredoxin) [1, 3, 6]. Under DCMU inhibition it is reasonable to suppose that, after repeated turnovers of P700, electron loss from the system could lead to loss of proper poise. Observations of electron flow through P700 under DCMU inhibition are not sufficient to test for occurrence of cyclic electron flow. Hence, I have sought to extend observations of the steady-state poise of P700 to see whether significant cyclic electron flow can be demonstrated or excluded.

Materials and methods

Measurements were made on three cyanobacteria: (1) *Agmenellum quadruplicatum*, strain PR6 (ATCC29404) [14], grown as previously described [11]; *Anacystis nidulans*, strain Tx20 (*Synechococcus* sp. PCC 6301 [14]) grown in medium C in continuous culture at 38 °C, 2% CO_2 in air, and white fluorescent illumination at a specific growth rate of about 0.7 $days^{-1}$; *Synechocystis* sp. PCC 6714 [14] grown in continuous culture in BG11 medium at 38 °C, 2% CO_2 in air, and white fluorescent illumination at a specific growth rate of 0.5 $days^{-1}$. I thank my colleagues J. Brand and J. Zhao for providing cell material of the latter two strains. The three strains chosen do not have heterocysts and are not nitrogen-fixers.

The two measuring systems have been described [11]. One measured O_2 exchange in a cuvette containing an inserted Clark-type electrode [12] and provided with controlled actinic light. The second system was a dual wavelength spectrophotometer in which P700 was viewed by weak measuring beams of 703 and 735 nm alternated at 120 Hz. Absorbance at a sensitiv-

ity of 1×10^{-2} full scale was observed alternately in darkness and in DC actinic light. (The sensitivity of 1×10^{-3} A full scale cited erroneously in the previous report [11] is hereby corrected.) Time periods chosen as sufficient to reach steady-state absorbance were 10 to 30 s light and 10 s dark. As an improvement for the present work the cuvette was thermostatted at 25 ± 0.5 °C.

The two measuring systems were provided with actinic light via two similar but not identical projectors. The projectors were controlled by shutters, limited in band pass by filter combinations (440, 620, 670, 680 nm) used interchangeably, and attenuated by a series of calibrated screens. In the course of the present work it became important to ensure that intensity measurements correctly compared quantum fluxes in the two cuvettes. Hence, this problem is treated in detail.

The spectrophotometer cuvettes were simple absorption cuvettes, usually 5×10 mm in section containing 1.0 cc and presenting 2.0 cm^2 to the actinic beam. The probe of a YSI Radiometer (a thermistor bolometer with 3 mm receiver) fitted interchangeably in the cuvette holder. The radiometer was calibrated via a thermopile against a NBS standard lamp to provide measurement of absolute intensity, corrected by a 0.95 transmission factor to the inside face of the cuvette. The O_2 electrode cuvette was made of 1.00 cm square glass tubing with a side opening for the electrode and was slightly tapered at the top to a 0.8 cm tapered glass plug with capillary opening; its volume was 2.0 cc. The electrode cuvette was contained within a rectangular-walled water jacket held at 25 °C. Intensities in the electrode cuvette were estimated by a 2×2 mm silicon photocell which could be positioned uniformly and interchangeably in either the electrode cuvette or the spectrophotometer cuvette. A further comparison of intensities in the two cuvettes was made by the chemical actinometer of Hatchard and Parker [5] adapted to use of a total volume of 2.0 cc of 0.15 M potassium ferrioxalate. Use of a Corning 5-60 filter with the 440 nm filter gave a band pass in which 96% of the energy was absorbed. Moles of ferrous ion formed in a timed exposure (20 to 60 s) were estimated by 1:10 phenanthroline and converted to Einsteins by the recommended quantum efficiency of 1.11 Einsteins/mole.

The model

The model is slightly modified and extended from the form used previously [11]. Let q designate the fraction of system 2 reaction centers (Q) in the oxidized (open) condition; let p designate the fraction of P700 in the reduced

(open) conditions. For any quantum flux, I, let A designate the fraction absorbed; then let α be the fraction of excitations available to Q and $(1 - \alpha)$ be the fraction available to P700. Let k_1 and k_2 be the probabilities that an excitation reaching an open reaction centre will cause it to transfer an electron. Then the rates of the two photoreactions are

$$v_1 = k_1(1 - \alpha)AIp \tag{1}$$

$$v_2 = k_2\alpha AIq \tag{2}$$

As viewed by P700 in cyanobacteria there is also another source of electrons provided by low potential donors (e.g., ferredoxin, NAD(P)H) via membrane carriers. In the literature electron flows of this type have been labelled respiratory and/or cyclic. Even though there are known modulating effects of light on respiration [10], the dark O_2 uptake rate will be taken as a benchmark measure of respiratory electron flow rate. A cyclic electron flow is not independently measurable. However, it should be possible to estimate the magnitude of the total *return* electron flow by its effect on the redox level of P700. In fact, that is the purpose of the model.

I consider two possible components of return electron flow. There may be one component so closely linked to photoreaction 1 that it is truly cyclic and can be evaluated as $r(k_1(1 - \alpha)AIp)$ where r is a fraction of the v_1 rate. A second component may be controlled by conditions of a redox pool, or kinetically buffered by other limiting reaction rates, so that it is linked to photoreaction 1 only indirectly and is not dependent on the prevailing v_1 rate. It will include all respiratory flow and possibly part or all of any "cyclic" flow. As a first approximation this second component will be treated as a constant and called R.

At steady-state the photoreaction 1 oxidation rate must equal the sum of the several reducing rates so that

$$v_1 = k_1(1 - \alpha)AIp = k_2\alpha AIq + rk_1(1 - \alpha)AIp + R \tag{3}$$

$$v_1 = k_1(1 - r)(1 - \alpha)AIp = k_2\alpha AIq + R \tag{3a}$$

which can be rearranged to give

$$p = \frac{k_2\alpha}{k_1(1 - \alpha)(1 - r)}q + \frac{R}{k_1(1 - \alpha)(1 - r)A} \cdot \frac{1}{I} \tag{4}$$

According to equation (4) it will be instructive to measure steady-state

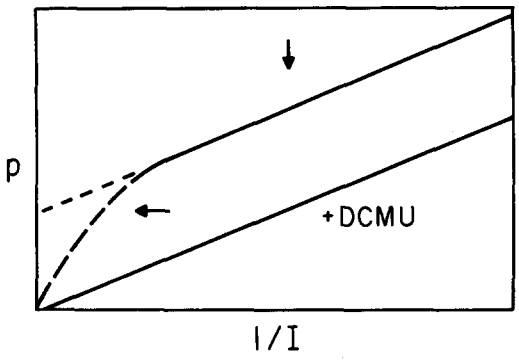

Fig. 1. Predictions of a model for photoreaction 1 of a cyanobacterium under varied intensity, I, of a light 1. p, the fraction of P700 in reduced form, is plotted versus 1/I. The linear segments have a slope, Ip, proportional to rate of photoreaction 1, and extrapolate to a intercept value of p which becomes zero in the presence of DCMU. A particular value of 1/I at which DCMU addition decreases p by a factor of 0.5x is called $1/I_c$.

values of p as a function of intensity of a light 1 (as 440 or 680 nm) both before and after addition of DCMU. Expected results are shown idealized in Fig. 1. In the presence of DCMU, q = O and the contribution of photoreaction 2 is eliminated. Then the p versus 1/I plot is linear, with a slope Ip, and extrapolates to zero. This is a statement of the constant Ip product observed under DCMU inhibition in the preceding paper [11]. In the absence of DCMU and at light-limiting intensities of a light 1, q is close to one and the first term becomes constant. The curve has the same slope observed with DCMU but now extrapolates to an intercept $k_2\alpha/k_1(1 - \alpha)(1 - r)$ which measures the relative photoreaction 2 contribution. At higher light intensities (cf. Fig. 2 of ref. 11) throughput electron rate becomes limited by the rate-limiting step between the photoreactions; then both q and p are driven toward lower values. Hence at low values of 1/I, p will depart from linearity in some fashion as proposed by the dashed line of Fig. 1.

The derivation of (4) and discussion of Fig. 1 contain all of the assumptions which will be used. One of these should be made explicit. The photoxidation rate, but not the reduction rate, of P700 is controlled by the fraction reduced. A different behavior was found by Hiyama et al [8, 9] in a study of P700 versus light intensity in chloroplasts and cyanobacterial membrane fragments. They provided NADP as a terminal electron acceptor and various concentrations of reduced 2,6-dichlorophenolindophenol as a donor. Their results (1/(1 − p) linear with 1/I) fulfilled the assumption that the reduction rate of P700 was proportional to (1 − p). The reason for the different assumption used for the present in vivo conditions is simply that p rather than 1(1 − p) usually approximated linearity with 1/I.

Results

General tests of the model

Characteristic plots of p versus 1/I for *Ag. quadruplicatum* are shown in Fig. 2A, B. In 620 nm (a light 2) the plot (not shown) was indeterminate (cf. Fig. 3 of ref. 11). After addition of DCMU the plot became similar to those for 440 and 680 nm with DCMU (Fig. 2A). At 440 and 680 nm there were characteristic linear segments which could be extrapolated to identifiable intercepts. On addition of DCMU the slopes were unchanged but the linear segments now extrapolated to a value of p close to zero. In the absence of

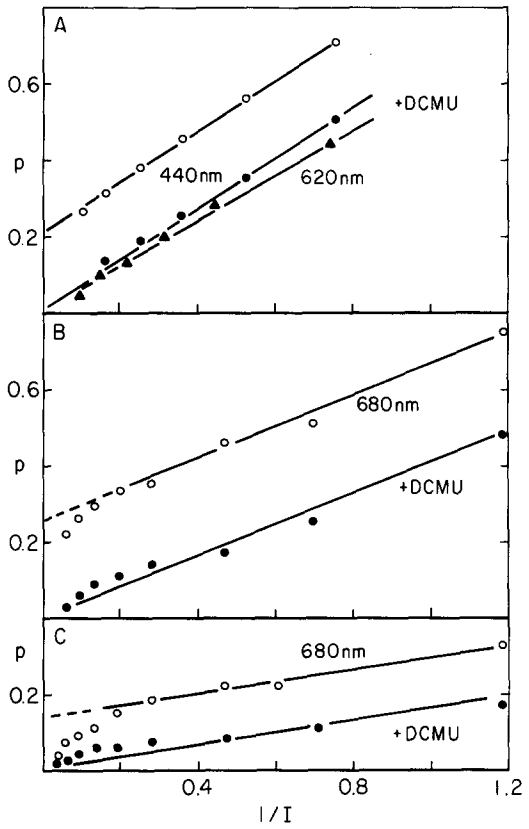

Fig. 2. Typical results found as tests of the model. p versus 1/I where I is in nE $cm^{-2} s^{-1}$. A. *Ag. quadruplicatum*, 4.5 μM Chl; ○●, with a 440 nm actinic light, ± 10 μM DCMU; ▲, 620 nm actinic light + 10 μM DCMU. B. *Ag. quadruplicatum*, 4.5 μM Chl, with 680 nm actinic light ± 10 μM DCMU. C. *A. nidulans*, 3.7 μM Chl, with 680 nm actinic light ± 10 μM DCMU.

Table 1. Comparison of values for I_c as estimated from v_{O_2} versus I and from p versus 1/I. The comparison is illustrated in Figure 5 and discussed in text.[a]

Λ, nm	From v_{O_2} versus I		From p versus 1/I	
	R	I_c	I_c	Intercept[b]
440[c]	9.9	1.4	2.9	0.17
440[c]	9.8	1.6	3.7	0.16
440[d]	6.9	1.0	3.1	0.15
440	6.0	0.9	2.4	0.14
680	6.9	0.5	1.6	0.25
680	9.0	0.5	1.7	0.26
680	6.0	0.4	1.2	0.21

[a] Cell concentrations were carefully matched in each set of experiments to give equal Chl/unit area, about 3×10^{-9} moles/cm^2.
[b] Extrapolated values of p for $1/I = 0$.
[c] Cells grown at 25 °C. For all other experiments reported cells were grown at 30 °C at which *Ag. quadruplicatum* grows more cleanly.
[d] From data plotted in Figure 5.

DCMU departure from linearity was observed at 680 but not at 440 nm. The difference is taken to be a trivial result of the facts that the intensities available at 680 nm, but not at 440 nm, were sufficient to reach light saturation (cf. Fig. 2 of ref. 11). Extrapolated intercepts for 440 nm were typically lower than for 680 nm (Table 1); the result implies that $k_2\alpha/k_1(1 - \alpha)$ is lower for 440 nm, i.e., that it is a better light 1. For a few experiments at 670 nm the extrapolated intercept was higher (> 0.3, data now shown).

Figure 2C shows a typical plot observed for *A nidulans* in 680 nm light. Extrapolated intercepts were typically smaller than for *Ag. quadruplicatum*, probably because *A. nidulans* has less allophycocyanim so that 680 nm is a better light 1 [13].

Effects related to changes in respiratory rate

Effects of dark starvation and of added glycerol on the dark respiratory rate of *Ag. quadruplicatum* have been documented (Table 1 of ref. 11) Effects on p versus 1/I plots are shown in Fig. 3A. Slopes of the linear segments typically decreased with dark incubation and then could be increased by addition of glycerol. Figure 3B shows a similar effect of added glucose for *Synechocystis* 6714, a demonstrated photoheterotroph [14]. Benzyl viologen, which apparently shunts electrons to O_2 via superoxide [15], lowers the slope of the p versus 1/I plot (Fig. 3C). All of the effects shown in Fig 3 are evident as changes in slope but without significant changes in extrapolated intercept. This is expected if the effects result from changes in the term R of (4).

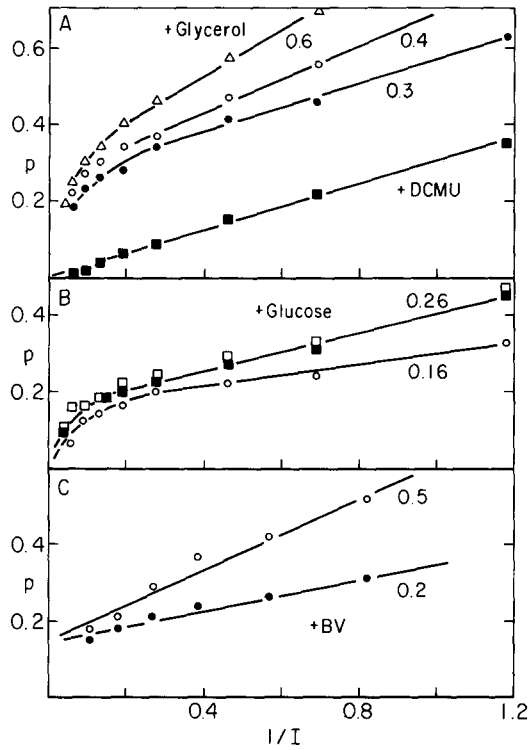

Fig. 3. Typical effects related to changes in respiratory rate p versus 1/I where I is in $nE\,cm^{-2}s^{-1}$. The number shown on each curve is an estimate of slope, Ip. A. *Ag. quadruplicatum*, 6.4 μM Chl with 680 nm actinic light. ○, 2 hr after harvest; ●, 6 hr after harvest; ■, 6 hr after harvest plus 10 μM DCMU; △, 6 hr after harvest plus 20 mM glycerol. B. *Synechocystis* 6714, 3.3 μM Chl, with 680 nm actinic light, ± 40 mM glucose. C. *Ag. quadruplicatum*, 2.6 μM Chl, with 440 nm actinic light ± 1 mM benzyl viologen.

Effects of cyclic mediators

Two of the well known mediators of cyclic phosphorylation in chloroplasts, phenazine methosulfate (PMS) and diaminodurene (DAD), were studied over a limited range of concentrations and conditions. Typical results obtained with *Ag. quadruplicatum* are shown in Fig. 4. In the range of 4 to 20 μM both cofactors had dramatic effects as increases (2 to 6x) in the slopes of p versus 1/I plots (Fig. 4A, B). Higher concentrations of 50 to 100 μM, as used in chloroplast studies [6], gave even greater slopes (Fig. 4A) but interpretation is clouded since p then approaches 100% before 1/I reaches a range where linearity is expected. The effects were not changed by anaerobic conditions maintained in the presence of DCMU (Fig. 4C, D). The

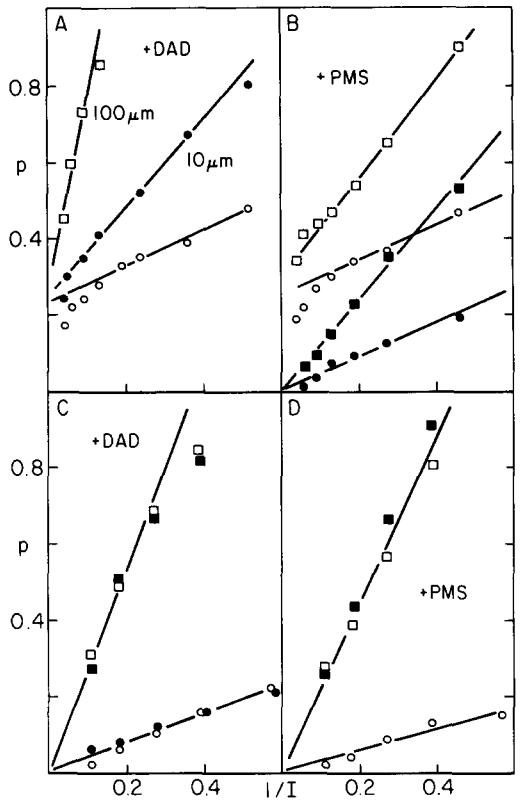

Fig. 4. Effects of diaminodurene (DAD) and phenazine methosulfate (PMS) on *Ag. quadruplicatum*. p versus 1/I where I is in nE $cm^{-2} s^{-1}$. A. 680 nm actinic light; ○, cells 2 hr after harvest; ●, plus 10 μM DAD; □, plus 100 μM DAD. B. 680 nm actinic light; ○, cells 3 hr after harvest; ●, plus 10 μM DCMU; □, plus 20 μM PMS; ■, plus 20 μM PMS plus 10 μM DCMU. C. 440 nm actinic light plus 10 μM DCMU; cells 3 hr after harvest under air (○) or argon (●); plus 20 μM DAD under air (□) or argon (■). D. 440 nm actinic light plus 10 μM DCMU; ○, cells 6 hr after harvest under air; plus 10 μM PMS under air (□) or argon (■). C and D were observed in 10 × 10 mm Thunberg cuvettes before and after 4x evacuation and degassing at 24 mm Hg with argon replacement. All other observations were in 5 × 10 mm cuvettes.

question of effects on the extrapolated intercept is not answered with certainty because of limitations in precision of measurement. Although in some experiments there appeared to be a small increase, it was never as large as the increase in slope. Effects of both mediators on *A. nidulans* showed the same features seen in Fig. 4.

Effects of both mediators were also evident in the O_2 exchange of *Ag. quadruplicatum*. DAD at 10 and 20 μM gave increases of about 30% in dark O_2 uptake. In limiting or saturating 680 nm light, DAD had no effect on net O_2 evolution at 10 or 20 μM but gave about 30% inhibition at 50 μM. PMS

at 10 or 20 μM gave 200 to 300% increases in dark O_2 uptake. In 680 nm light effects of PMS on O_2 evolution were more complex: 10 μM had no effect at low intensity but gave about 50% inhibition at high intensity; 50 μM gave 50% or greater inhibition at both high and low intensity.

Complexities in effects on O_2 exchange superimposed on the complicating dark decay in O_2 uptake led me to treat these in vivo effects of PMS and DAD as a separate problem for future work. Evident differences in effects of PMS and DAD imply that they have different sites of action, as expected from chloroplast studies [6]. However, they have in common the effect of greatly increasing the rate of photoreaction 1 by increasing the return rate of electron supply. Apparent lack of effect on the extrapolated intercept could be taken as evidence that the increased return electron flow is described by the R term and not by the r term of the model. However, this may be a trivial result. Both cofactors were used at purposely low concentrations so that effects might be seen at lower intensities where v_2 is linear with intensity. Hence the rate effects observed may be concentration-limited and dependency on light intensity and the v_1 rate may be obscured.

Comparisons of I_c estimated via rate O_2 evolution versus I and via p versus 1/I.

Because of the suppression of respiratory O_2 uptake by light (Kok effect) cyanobacteria do not show a classical compensation point in a light 1. However, it is still possible to define operationally a special value of intensity, Ic, at which the rate of O_2 evolution is equal to the rate of dark O_2 uptake. *If dark O_2 uptake is taken as a measure of R*, then I_c is the intensity at which $v_2 = -R$ as viewed by O_2 exchange. I_c also should be measurable via photoreaction 1, to which both v_2 and R are additive contributors. As viewed by photoreaction 1, I_c should be the intensity at which v_2 and R are equal and additive (Equation 3a). Hence at I_c addition of DCMU should abolish v_2 and reduce the rate v_1 by a factor of 0.5. On a p versus 1/I plot the value of 1/I at which addition of DCMU decreases the value of p by 0.5x is taken to be $1/I_c$.

In the previous report [11] it appeared that identical values of I_c were estimated from a plot of the rate of O_2 evolution versus I and a plot of p versus 1/I. Attempts to repeat this observation led to divergent values of Ic and subsequent work led to recognition of complexities.

First, there is a problem of equivalence of intensities in differently designed cuvettes and in two different projection systems. This was examined by side-by-side comparisons of intensities with a silicon photocell and with a chemical actinometer (Table 2). Intensities estimated by the actinometer

Table 2. Comparison of actinic light intensities delivered to cuvettes by two projection systems.

Cuvette		Incident quantum rate[a], $nE\,s^{-1}$	
		Actinometer[b]	Radiometer, Silicon cell[c]
Spectrophotometer		8.09	9.92
		8.00	10.41
		7.67	10.41
	av.	7.9	10.2
Oxygen electrode		10.67	11.76
		10.06	12.80
		10.62	12.88
	av.	10.5	12.5

[a] All measurements made at 440 nm, for areas taken as 2.00 cm^2, and with lamps of the projection systems held at 80.0 ± 0.05 volts.
[b] Described in Materials and methods.
[c] Measurements for the spectrophotometer cuvette were made by a YSI radiometer with a 3 mm circular receiver. Measurements in the oxygen electrode cuvette were made by a 2 × 2 mm silicon cell which was calibrated against the YSI radiometer in the spectrophotometer cuvette.

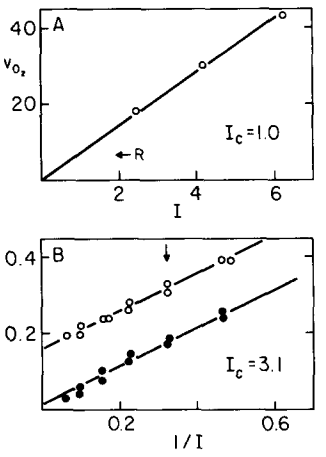

Fig. 5. Estimation of I_c via O_2 evolution and p versus 1/I; one data set. A. Rate of O_2 evolution, $O_2\,Chl^{-1}hr^{-1}$, versus I, $nE\,cm^{-2}s^{-1}$ of 440 nm, for cells 2 to 4 hr after harvest, 2.85 mM Chl, in a 2 cc cuvette of path length 1 cm. I_c is estimated as the intensity needed to give O_2 evolution at a rate equal to the dark O_2 uptake of 6.9 $O_2Chl^{-1}hr^{-1}$. Rates of O_2 evolution shown are those observed in light and without any correction for respiration. B. p versus 1/I, 440 nm actinic light, cells 4 to 5 hr after harvest, 1 cc in a 5 × 10 mm cuvette with 5 mm actinic path length, 5.7 mM Chl; with (●) and without (○) 2 mM DCMU. I_c is taken from the value of 1/I at which addition of DCMU reduces p by a factor of 0.5x.

were about 0.8 of those estimated via the silicon cell and referenced to the radiometer. I attribute the difference to non-uniformity of projection beams and a higher intensity sampled by the 4 mm^2 area of the silicon cell positioned in the center versus the total area (200 mm^2) sampled by the actinometer. The somewhat higher fraction measured by the actinometer in the electrode cuvette probably arises from a slightly larger area presented by the tapered cuvette. I take the results of Table 2 to show that there are no large discrepancies in estimates of intensities made via the silicon cell in the two cuvettes.

Comparisons of I_c values also contain the more difficult problem of assessment of a proper value for the respiration rate which can be measured only as dark O_2 uptake. Although the model takes R as a constant, this can be true only under narrowly defined conditions. After harvest from a turbidostat culture the respiration rate of *Ag. quadruplicatum*, measured on samples held in darkness, decays rapidly during the first three hours [11]. (A similar dark decay in a thermophilic *Synechococcus* species has been described [7].) The sets of measurements reported in Fig. 5 and Table 1 were made after several hours dark incubation under 2% CO_2 in air and estimates of respiration were made following low light intensities of 440 or 680 nm.

Table 1 gives seven sets of values of I_c taken from experiments such as that shown in Fig. 5. The averaged result is that the value of I_c estimated by the model for photoreaction 1 is almost 3x larger than the value estimated from O_2 exchange. This implies that the value of R, the total return electron flow to photoreaction 1, is almost 3x larger than the value measured as dark O_2 uptake. It is not clear whether this result has fundamental meaning or whether it is a trivial result of different conditions of measurements In terms of O_2 exchange, I_c was estimated from the slope of the light intensity curve and the rate of dark O_2 uptake measured between 2 and 5 minutes after light exposures In terms of p versus 1/I, I_c was estimated from measurement of the steady-state value of p obtained by alternated periods of 10 to 30 s light and 10 s darkness. If there is any decay in respiratory rate within the first two minutes after a light 1 exposure, then its rate as measured in terms of O_2 exchange will be underestimated.

Uncertainties about factors controlling respiration rate limit the conclusions which can be drawn from values of I_c and R as viewed by photoreactions 1 and 2. The results allow the possibility that there is a special component of return electron flow, as from NAD(P)H, up to 2x the rate of respiratory flow. Alternatively, one may suppose that the respiratory electron flow is entirely homogeneous but subject to a 3x increase in rate under a low light 1.

Discussion

My central thesis is that any cyclic electron flow around photoreaction 1 should be evident in the poise of P700. In light 1 illumination, a condition expected to be favorable for cyclic flow [3], the rate of photoreaction 1 is limited by the rate of total electron flow to P700. For this condition a simple model predicts that the steady-state fraction (p) of P700 reduced should be linear with the reciprocal of intensity (1/I). The model provides a reasonable fit to observations on three cyanobacteria.

A possible component of cyclic electron flow can be treated as having some fraction, r, of the photoreaction 1 rate. Such a component should be identifiable by its effect as a factor $1/(1 - r)$ operating on both the slope and extrapolated intercept of a p versus 1/I plot. Inspection of equation 4 shows that the terms $(1 - r)$ and k_1 have equivalent effects. Although purposely introduced to label one possible form of return electron flow, r is redundant; it merely reduces the throughput efficiency of photoreaction 1 and its effect could be included in the meaning of k_1 [18].

The question must be asked whether values of the extrapolated intercept, as in Fig. 2, can accomodate a significant r component. A minimum possible value of the intercept is established by the ratio $k_2\alpha/k_1(1 - \alpha)$ which will be increased by $1/(1 - r)$ if r is greater than zero. The terms k_1 and k_2 are limiting quantum yields and probably close to one. So the minimum intercept is established by the ratio of distribution of excitations to the two photoreactions, $\alpha/(1 - \alpha)$. In their study of action spectra of photoreactions 1 and 2 in *A. nidulans* Wang et al [19] estimated a value of 0.18 for α at 680 nm. Hence a predicted minimum intercept, $\alpha/(1 - \alpha)$, is 0.22. If there is a significant component describable as r, then the intercept should be greater than 0.22. However, observed values (cf. Fig. 2C) were somewhat lower (average about 0.16). This comparison ignores second order effects like state changes and growth conditions but argues against any large endogenous r component.

A characteristic feature of p versus 1/I plots was that the slopes were unchanged by addition of DCMU. The slope must include the effect of any cyclic flow as in the factor $1/1 - r$ (equation 4). Hence any r component must work equally in the presence or absence of DCMU. This raises the problem of how proper poise for continued cyclic flow [1, 3, 6] could be maintained in the presence of DCMU. One might propose that poise can be maintained by the R component. However, it is evident that the lack of effect of DCMU on the slope places an added constraint on the possibility of cyclic electron flow via a component such as r.

Another possible component of cyclic electron flow, which is independent

of prevailing light intensity, will operate upon P700 in a fashion similar to that of respiratory electron flow. Hence, it is convenient to consider a total return flow, R, and then ask whether R is significantly greater than can be attributed to respiratory flow.

Treatment of R as a constant proved satisfactory over the limited time period needed to obtain a set of data. Over longer time periods there is a dark decay in slope which parallels dark decay in respiratory O_2 uptake. Further, both the slope and the dark O_2 uptake could then be increased by added substrates (Fig. 3). The total range of variability in slope of p versus 1/I was about 3x. However, all the dark O_2 uptakes observed were less than the rate previously reported [11], ($15\ O_2 Chl^{-1} hr^{-1}$) which is only 5% of the light saturated O_2 evolution rate in *Ag. quadruplicatum* at 25 °C.

Three salient arguments now may be summarized:

(1) Under a light 1 the relative rate of photoreaction 1, Ip, is small compared to its potential rate because p is held at low values by limiting electron input. Nevertheless, changes in respiratory rate induced by starvation or substrate addition make concomitant changes in Ip. Hence any additional return electron flow cannot be large compared to respiration (Fig. 3).

(2) Addition of PMS or DAD, known mediators of cyclic electron flow and cyclic phosphorylation in chloroplasts, cause large (up to 5x) increases in the rate of photoreaction 1 as seen in the slope of p versus 1/I plots (Fig. 4). Compared to such facilitated cyclic flow, any endogenous cyclic flow must be small and with a low quantum yield limited by low values of p.

(3) Respiratory rate and rates of photoreactions 1 and 2 were compared via a light intensity compensation point, I_c (Fig. 5 and Table 1). The comparison shows that the total return rate is not more than 3x the respiratory rate measured as dark O_2 uptake. The comparison is made at low intensity. However, in none of the many p versus 1/I plots is there any discontinuity which would suggest a higher proportion of cyclic flow at higher intensities.

From these arguments and the lack of any contrary evidence in the data, I conclude that cyclic electron flow and any dependent phosphorylation are not significant processes in these cyanobacteria under ordinary light intensities.

I find this a monstrous conclusion. Demonstrations of cyclic phosphorylation in chloroplasts, even mediated by very low concentrations of added ferredoxin, naturally leads to the expectation that cyclic phosphorylation is a natural and significant in vivo event [3, 6]. Further, concerns about providing for the high ATP demands of microbial cell synthesis lead to expectation that need for cyclic phosphorylation should be even greater in

cyanobacteria than in chloroplasts [10]. Such thoughts lead to doubts about my conclusion and whether it is merely an anomaly in search of explanation. In any event the present work directs attention to necessary effects of cyclic electron flow upon photoreaction 1, a problem too long neglected.

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