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ON THE STATE 1–STATE 2 PHENOMENON IN PHOTOSYNTHESIS

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SUMMARY

The State 1–State 2 phenomenon of photosynthesis was studied in *Chlorella* by measuring the flash yield (Y) and the modulated rate (v) of oxygen evolution induced by weak modulated 650-nm light. From light intensity curves, intensities of 650 and 710 nm background and preilluminations were chosen to give maximum values of Y and v. Following long preilluminations in 710 nm (State 1) or in 650 nm (State 2), Y and v were measured in background light of chosen wavelength. The resulting plots of v vs Y show a discontinuity between State 1 and State 2. They confirm the predictions of Bonaventura and Myers [(1969) Biochim. Biophys. Acta 189, 366–383] and are consistent with changes in α (fraction of absorbed light captured by System II) as explanation of the State 1–State 2 phenomenon. When the intensity of 710 nm preillumination was too low, the characteristics of State 1 were not fully developed and the results then were similar to those of Delrieu [(1972) Biochim. Biophys. Acta 256, 293–299].

INTRODUCTION

In *Chlorella* there is demonstrable what has come to be known as the State 1–State 2 phenomenon. At low light intensities chosen to give maximum quantum yield of photosynthesis, there are two different steady states. State 1 develops after prolonged (min) illumination in a Light 1 (as 710 nm); it is characterized by an increased efficiency in use of Light 1 and a decreased efficiency for Light 2. State 2 develops after prolonged illumination in a Light 2 (as 650 nm); it is characterized by an increased efficiency in use of Light 2 and a decreased efficiency for Light 1. Because of the reciprocal characteristics of the two states, Bonaventura and Myers [1] proposed that change in state is caused by change in distribution of excitation energy to the two photoreactions. Essentially similar conclusions were reached by Duysens [2] and Murata [3] in his study of fluorescence in *Porphyridium cruentum*.

Bonaventura and Myers [1] made an explicit prediction with respect to Joliot and Joliot [4] plots of the function

Abbreviation: LED, light emitting diodes.

$$v = k\alpha I \frac{q}{1-p(1-q)} \quad (1)$$

Here v is rate of oxygen evolution, I is rate of quantum absorption, α is the fraction of excitation energy delivered to centers of Photoreaction 2, k is the quantum yield of Photoreaction 2, q is the fraction of open centers for Photoreaction 2, and p is the Joliot probability factor. They predicted that plots for State 1 and State 2 would differ only in the value assigned to α for any wavelength of actinic light.

Delrieu [5] examined the above prediction with essentially negative results. At 650 and 680 nm she could observe values of v and q (flash yield) that were higher in State 2 than in State 1. However, all values were close to a single plot of v vs q and there was no evidence for change in α. Furthermore there was no difference between states as viewed by v and q in 705 nm. In short, Delrieu failed to observe one-half of the phenomenon described by Bonaventura and Myers.

We were surprised by Delrieu's results and also by our own initial failures to observe the phenomenon as originally reported (ref. 1). Further study showed that State 1 is intensity dependent and not completely developed in darkness. We found that rather high intensities in the mW/cm^2 range at 710 nm are needed to obtain maximum quantum yield and flash yield. We are led to suspect that errors have been made in past work in that intensities at 700 to 710 nm have been too low to randomize the S states to $S_3 = 0.25\,\Sigma S_i$ (Kok et al. [6].)

We present data on intensities of actinic light at 650 and 710 nm as viewed by oxygen flash yield and by rate of oxygen evolution from a low intensity modulated beam. Then we examine the State 1–State 2 phenomenon and consider effects of intensity thereon.

METHODS

Chlorella pyrenoidosa (Emerson strain) was grown, harvested, and treated as previously described (Wang and Myers [7]). The final preparation gave a layer of selected small cells not more than one cell thick on a bare platinum rate-measuring electrode maintained at about 25 °C. The optical system (ref. 1) provided two actinic beams chopped at 80 Hz plus the beam from a xenon tube giving 10-μs flashes (ref. 7). The modulated rates of oxygen evolution produced by the actinic beams will be called the background rate, V, and the flash yield will be called Y. An additional modulated light source of constant intensity was provided by an array of 4 light emitting diodes (LED, Fairchild FY-102) positioned symmetrically above the electrode. The LED array was pulsed at 14 Hz and 10 mA to give 650 nm emission at half-band width of 25 nm and an intensity equivalent to 5 μW/cm^2 of 650-nm actinic light. The modulated rate induced by the LED will be called the probing rate, v.

Electrical circuit of the electrode (ref. 7) was designed to minimize ohmic resistance and linearly convert electrode current to output voltage. Bias was provided to suppress the DC level. Feed-back elements of 10 kΩ and 0.001 μF gave a low pass filter (t_c 10 μs) and a sensitivity of 10 mV/μA. Output voltage was recorded on three instruments. (a) A Brush (Mark 220) recorded dc rate and flash yield via a low pass filter of t_c 12 ms. (b) A lock-in amplifier (PAR Model HR-8, Type A) measured the modulated rate (v) induced by the 14 Hz LED. (c) A second lock-in amplifier (PAR

Model HR-8, Type B) via a unit gain AC amplifier measured the modulated background rate (V) induced by either of the 80-Hz actinic beams. The lock-in amplifier outputs were read on a dual pen recorder.

Actinic light intensity was measured by a calibrated silicon photocell. The measured peak-to-peak intensity is used throughout this report without correction for reflection from the platinum surface (which increases fractional absorption by a factor of about 1.5) and without dividing by 2 to obtain time-averaged intensity. Background rate (V) and saturating flash yields could be compared by the following procedure: actinic light or repetitive flashes at 5 flashes per s were turned on-off for a few 10-s cycles with a 1-s time constant on the Brush recorder. Peak-to-peak changes in each cycle allowed conversion of the dc rate component to an equivalent number of flashes per s.

RESULTS AND DISCUSSION

Fig. 1 shows the background rates (V) of oxygen evolution, relative quantum yields (V/I) and probing rate (v) as functions of actinic light intensity (I) at 650 and

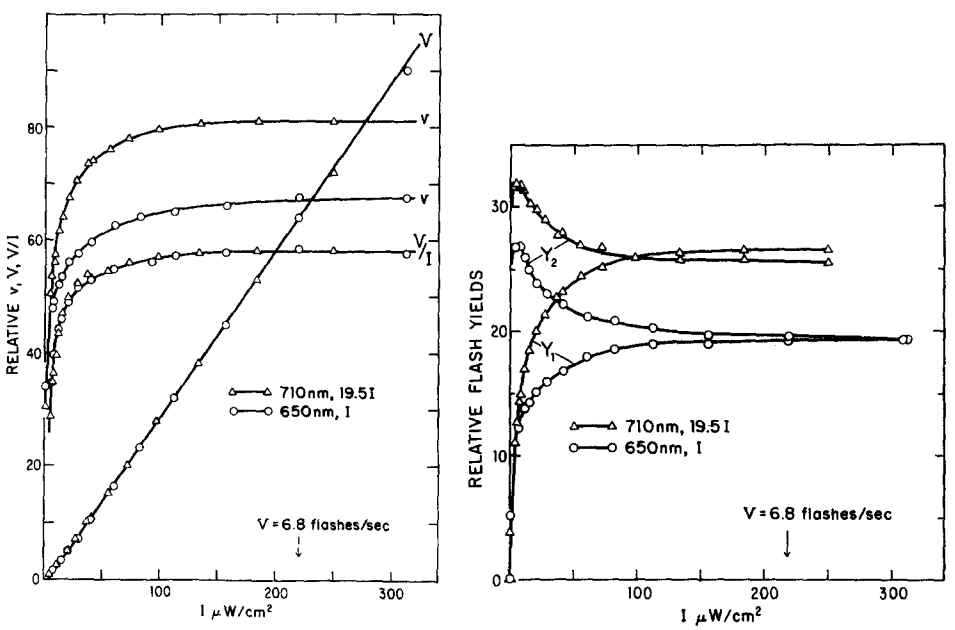

Fig. 1. Effect of 710 and 650 nm background intensity on rate (v) of oxygen evolution in response to modulated low intensity 650 nm probing light. Relative rate (V) of oxygen evolution in response to background light was measured in the absence of probing light. Background light was modulated at 80 Hz and probing light at 14 Hz. The intensity of probing light was estimated as equivalent to 5 μW/cm^2 of 650 nm background light. All intensities are taken directly from peak-to-peak measurements with no further correction. A vertical arrow shows that repetitive flashes at 6.8 flashes per s give a dc-measured photochemical rate equivalent to I 220 μW/cm^2.

Fig. 2. Effect of 710 and 650 nm background light on oxygen yields from the first (Y_1) and the second (Y_2) of a series of flashes spaced at 0.2 s. The data were obtained from the same preparation used for Fig. 1.

710 nm. Both V and v are measured in relative and not identical units. Background rates in both wavelengths can be normalized to a single curve if intensity at 710 nm is divided by 19.5. We shall refer to the normalized values of I. A vertical arrow shows that 220 μW/cm^2 gives a dc-measured photochemical rate equivalent to that of repetitive flashes at 6.8 flashes per s.

Fig. 2 shows relative peak heights of flash yields for the first two (Y_1, Y_2) of a series of saturating flashes spaced at 0.2 s on the same background intensities and with the probing light off. Variations in Y_3 and Y_4 (not shown) were smaller. At $>$ 200 μW/cm^2 each of the first four flash yields were 0.25$\pm$0.01 of their sum.

The data of Figs 1 and 2 are expected results of the deactivation of the S states at the oxidized side of Photoreaction 2 (Kok et al. [6]). They are also reasonably consistent with the more direct and precise observations of Lemasson and Barbieri [8] for half times in decay of Y_1 of 3.8 s after 650 nm to 4.8 s after 710 nm in *Chlorella*. The essential feature demonstrated is that a relatively high intensity, about 2 mW/cm^2 of 710 nm is required to randomize the S states and give maximum quantum yield and Y_1 flash yield. It was necessary to use a 2500-W xenon arc to obtain sufficient intensity at 710 nm through our Bausch and Lomb monochromator.

Fig. 3 shows the tracing from a record which demonstrates a typical protocol of our further experiments. Intensities of background light were chosen to give maximum quantum yield and Y_1 flash yield and approximately equal rates of oxygen evolution (V). Fast changes in v are ascribed to changes in fraction of open reaction centers; slow changes describe a part of the State 1–State 2 phenomenon. Flash yields (obtained from separate recordings for single flashes) and values of v were taken as noted by the arrows. State 1 values were estimated after long time ($>$5 min) in 710 nm and 15 s after transition to 650 nm (or other chosen wavelength λ). State 2 values were estimated after long time ($>$ 5 min) in 650 and 15 s after transition to 710 nm (or other chosen wavelength λ). The long-time background light (used to establish the state) will be called the preillumination and the background light used during

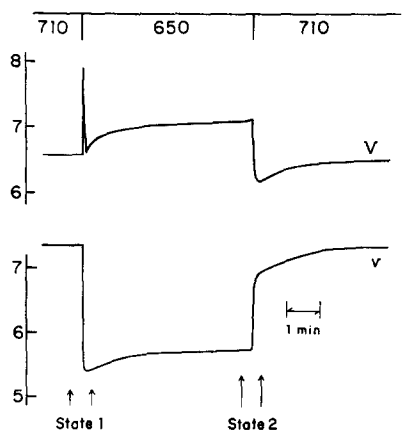

Fig. 3. A typical record showing experimental protocol for State 1 and State 2 measurements. V designates rate in response to background lights modulated at 80 Hz and at 710 or 650 nm as noted. v designates rate in response to the 650 nm probe modulated at 14 Hz. Rate v and flash yield Y (from a separate record not shown) were estimated at times noted by arrows.

TABLE I

FLASH YIELD (Y) AND MODULATED RATE (v) MEASURED IN 650 AND 710 nm FOR STATE 1 AND STATE 2

A, time course of transition between states. B, average values with standard deviations from five experiments for State 1 and State 2. Intensities in $\mu W/cm^2$ for 710 nm were 4000 in A, 4000 to 7000 in B; for 650 nm intensities were 400 in A, 300 to 600 in B.

A. Transition:	State 2 → State 1			State 1 → State 2	
t in 710 nm	Y	v	t in 650 nm	Y	v
15 s	0.99	0.90	15 s	0.45	0.73
1.5 min	0.99	0.95	6 min	0.65	0.82
5 min	0.99	0.98	13 min	0.70	0.83
9 min	1.00	1.00			
B. Average values:	in 710 nm		in 650 nm		
State 2	0.974	0.884	State 2	0.690	0.794
< 15 s	±0.015	±0.015	> 10 min	±0.076	±0.027
State 1	1.000	1.000	State 1	0.466	0.716
> 9 min			< 15 s	±0.018	±0.015

estimation of v and Y will be called λ.

Table IA presents estimates of probing rate v and flash yield Y at various times during the transition between states as established by intensities of 650 and 710 nm chosen to give maximum values (cf. Figs 1 and 2). Our average values (Table IB) are significantly different than those reported by Delrieu [5]. We observe a probing rate v in 710 nm which is significantly lower for State 2 than for State 1. In 650 nm we observe, as Delrieu, values of v and Y lower in State 1 than in State 2.

Fig. 4 presents one of several experiments in which fraction of open centers (q) was varied by wavelength of background light after a preillumination to establish State 1 or State 2. The v vs Y plot shows discontinuity between State 1 and State 2 and supports the prediction of Bonaventura and Myers [1] that differences between states can be explained as change in α of about 10%.

When the preillumination light was chosen at lower intensities, then the discontinuity between State 1 and State 2 disappeared, all values for v and Y (as fractions of values for 710 preillumination) became larger, and the resulting plot (not shown) was similar to that of the single curve observed by Delrieu [5]. Darkness or low intensities of 710 nm preillumination did not completely establish State 1 in giving maximum values for v and Y observed at 700 (or 710 nm). However, darkness and all intensities of 710 nm preillumination are indistinguishable in giving the minimum values of v and Y observed in 650 nm.

In search of additional evidence we repeated the experiment reported by Duysens [2] on the effects of State 1 vs State 2 on the oxygen yield of an attenuated flash. We measured flash yields on 710 nm background after preillumination with 710 nm (State 1) and after preillumination with various intensities of 650 nm (State 2). We chose a saturating flash (as used in other experiments) with flash energy sufficient to give 6 quanta per System II reaction center and near-maximum oxygen yield (ref.

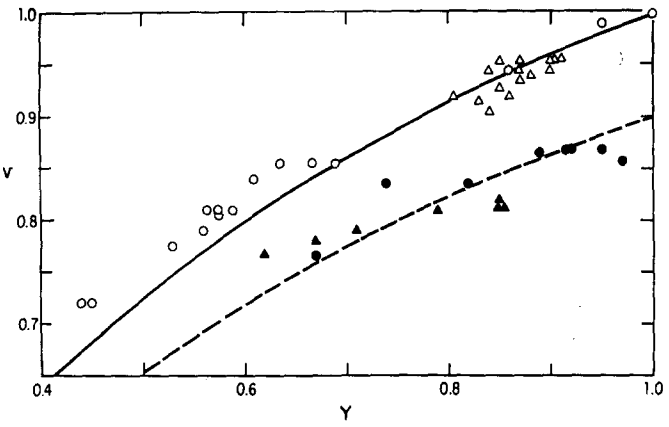

Fig. 4. Probing rate (v) vs flash yield (Y). State was established by long preillumination, 6600 μW/cm^2 of 710 nm for State 1, 300 μW/cm^2 of 650 nm for State 2. Observations were made under background light of wavelengths (λ) chosen to vary fraction of open centers. State 1 values were observed under λ (○) or $\lambda+710$ nm (△) where λ was varied between 580 and 710 nm. State 2 values were observed under λ (●) or under $\lambda+650$ nm (▲) where λ was varied between 650 and 710 nm. All values of v and Y are fractions of values observed in 710 nm after 710 nm preillumination. Curves are drawn for a Joliot probability factor $p = 0.62$ and for $\alpha/\alpha_{max} = 1.0$ (—) or $\alpha/\alpha_{max} = 0.9$ (– – –). Intensities for preilluminations and λ were chosen to give approximately equal rates (V) of oxygen evolution.

7). We chose an attenuated flash energy which gave about 1/4 of the maximum flash yield. The results are given in Table II. The saturated flash yield was essentially independent of state. With sufficient intensities of 650 nm used to develop State 2, the attenuated flash yield was about 8% lower in State 2 than in State 1. Evidently the absorption cross section for System II reaction centers is indeed higher in State I than in State 2.

Our essential finding is that darkness is not entirely equivalent to a Light 1 in developing State 1. As viewed by a Light 2, darkness and Light 1 preillumination appear equivalent. However, as viewed by a Light 1, darkness and Light 1 preillumination are not identical. Our results and those of Delrieu become compatible if we assume that her use of darkness or too low intensity preillumination at 705 nm

TABLE II

EFFECT OF STATE ON YIELDS (Y) OF SATURATING AND ATTENUATED FLASHES

Flashes were given on a background of 4500 μW/cm^2 of 710 nm after 6 to 15 min in 710 nm (State 1) or 15 s after a 6 to 20 min preillumination in 650 nm (State 2) at the intensity shown. In State 1 the attenuated flash yield was 0.26 of the saturating flash yield.

650 nm preillumination (μW/cm^2)	(Y, State 1)/(Y, State 2)	
	Saturating flash	Attenuated flash
640	1.00	0.92
420	0.98	0.91
320	0.99	0.91
165	0.97	0.92

did not give maximum values of v and Y.

An important feature of our results is that State 1 and State 2 have reciprocal effects on rate and flash yield observed at 650 nm and at 710 nm (Table IB, Fig. 4). It was this characteristic reciprocal effect which led Bonaventura and Myers [1] to suggest explanation in terms of changes in α, the distribution of excitation energy between the photosystems. Because she observed only one-half of the total phenomenon, Delrieu [5] was able to propose a different explanation in terms of a variable apparent equilibrium constant (K) between the photoreactions. Although Delrieu's proposal is a priori more plausible, it will not alone account for the reciprocal effects. We conclude that our findings are consistent with, though they do not prove, the hypothesis of changes in α as an explanation of the State 1–State 2 phenomenon. Our work contains no information on mechanism by which changes in α might be made.

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