BBA 46273

CHANGES IN OXYGEN EVOLUTION INDUCED BY A LONG PREILLUMINATION AT 650 nm WITH *CHLORELLA PYRENOIDOSA*

MARIE-JOSÉ DELRIEU
Laboratoire de Photosynthèse, C.N.R.S., 91-Gif-sur-Yvette (France)
(Received September 2nd, 1971)

SUMMARY

The relationship between the rate of O_2 evolution and the corresponding concentration of System II active centers E has been studied in "State 1" and in "State 2". No variation of α (fraction of absorbed light captured by System II) could be observed. The phenomena shown by BONAVENTURA AND MYERS (*Biochim. Biophys. Acta*, 189 (1969) 366) may be interpreted by an increase of the apparent equilibrium constant between the two photoreactions II and I when the algae are illuminated at 650 nm.

INTRODUCTION

BONAVENTURA AND MYERS[1] have shown a change in the rate of O_2 evolution associated with a change of the fluorescence yield during a long illumination at a wavelength where System II absorbs notably ("Light 2":650 nm, for example). These authors have called "State 1" the state of algae after darkness or after a preillumination at a wavelength essentially absorbed by System I ("Light 1":705 nm, for example) and "State 2" the state of algae after a long Light 2 preillumination. To explain the phenomena which they observed, they proposed that Light 2 brings about a decrease of α (that is the fraction of absorbed quanta of a given wavelength delivered to System II) by affecting pigment distribution.

In ref. 2, we made some remarks in agreement with this hypothesis. However, we decided to submit this hypothesis to a supplementary experimental test by measuring a parameter that was lacking in the experiments of BONAVENTURA AND MYERS: the relationship between the rates of O_2 evolution and the concentration of active centers of System II, E (measured by the O_2 "microburst" brought about by a short flash of saturating light).

METHOD AND MATERIAL

O_2 exchange was measured using a concentration-type bare electrode as described in ref. 2 with the addition of an electronic flash. Experiments were performed with *Chlorella pyrenoidosa* in the culture medium (200 μg chlorophyll per ml) at 20°. The algae were grown as previously described[2].

EXPERIMENTS, RESULTS AND DISCUSSION

We have studied the relationship between the rate of O_2 evolution (v_{O_2}) and the corresponding concentration of E in State 1 and State 2. As shown by JOLIOT[3], the relationship is not linear:

$$v_{O_2} = k_{II}\alpha_i i \frac{e}{1 - p + ep} \quad (1)$$

i.e. the rate of O_2 evolution depends on three factors, i (the absorbed light intensity), α and e (relative concentration of E measured by the flash); k_{II} is the quantum yield of Photoreaction II and p, the probability factor of energy transfer between System II traps ($\simeq$ 0.5). This relationship is drawn on Fig. 1.

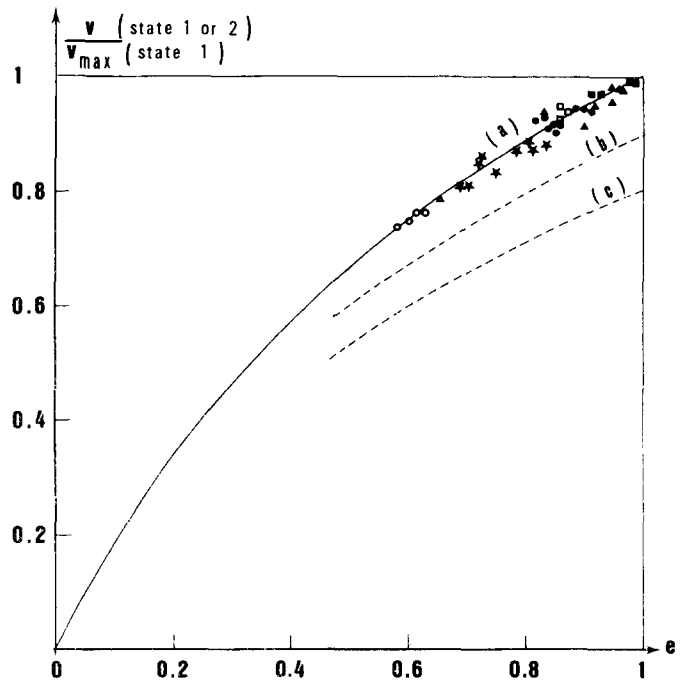
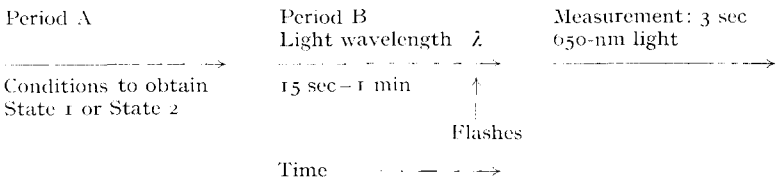


Fig. 1. Curve v/v_{max} as a function of E in State 1 and in State 2. Open symbols (○, △, □) in State 1, closed symbols (●, ▲, ■) in State 2; ○ ●, at 650 nm; △ ▲, at 680 nm; □ ■, at 690 nm; ★, data obtained for 680 nm during a long 680-nm preillumination. The same maximum values of e and v/v_{max} were obtained at 705 nm in State 1 and 2. a, curve if there is no variation of α ($\Delta\alpha = 0$); b, curve if $\Delta\alpha = 0.1$; c, curve if $\Delta\alpha = 0.2$. Chlorella, 200 μg chlorophyll per ml. 20°, air.

The experimental measurements were carried out in the following manner. State 1 was obtained by keeping the algae in darkness or preilluminating them with low-intensity 705-nm light, in both cases during about 10–30 min. Then, after a rather short illumination (15 sec to 1 min) of different wavelengths (650, 680, 690 and 705 nm), giving equal rates of O_2 evolution, E was measured by saturating flashes and the rate of O_2 emission was obtained by substituting to the continuous

illumination a reference light at 650 nm for 3 sec. For State 2, before applying the irradiation above described, we preilluminated the algae for 10 min, at 650 nm with a light in the same range of energy as that used by BONAVENTURA AND MYERS[1] (2500 ergs·cm^{-2}·sec^{-1}) or slightly higher (up to 5000 ergs·cm^{-2}·sec^{-1}). This schedule is summarized below:



The value of e is predetermined by Period B and therefore we measure:

$$v_{O_2} = k_{II} \cdot \alpha(650\ \text{nm}) \cdot i(650\ \text{nm}) \frac{e(\lambda)}{1 - p + e(\lambda)p} \qquad (2)$$

where λ is the wavelength (650, 680, 690 and 705 nm) in Period B.

So the differences between the rates depend only on the concentration e (λ), which has no time to change during the 3 sec of illumination at 650 nm (ref. 3), and on α, only in the hypothesis where α (650 nm) varies between State 2 and State 1.

v_{max}, the maximum value of v_{O_2} (see Fig. 1) is obtained when $e \simeq 1$, *i.e.* after illumination with Light 1 ($\lambda = 705$ nm) and in State 1.

BONAVENTURA AND MYERS explain their results by a relative decrease of α of 0.1; if this is true, we should obtain similar curves $v/v_{max} = f(e)$ (Eqn. 1) in State 1 and in State 2, but in the latter case v/v_{max} decreased to a ratio 0.9 of that obtained in the former (see Curve b in Fig. 1). The experimental points in Fig. 1 show that this is not true.

We find that for each wavelength, during the transition from State 1 to State 2, the values of v/v_{max} and e increased and moved along the same curve already found by JOLIOT[3] in State 1. The values in State 2 of v/v_{max} and e are closer to 1 when the intensity of the preillumination light at 650 nm is increased.

All these results prove that Eqn. 1 remains true in both State 1 and 2 where there is no variation of $k_{II}\alpha$, as shown above, and of p. (A variation of p would affect the curvature of the curve: for example if $p = 0$ the curve is a straight line.) We find the same rate of O_2 evolution and the same E concentration at 705 nm in State 1 and in State 2 (see Table I). Apparently this result is different from that described by

TABLE I

VALUES OF THE STEADY-STATE RATE OF O_2 EVOLUTION v_{O_2} AND E AT 705 nm IN STATE 1 AND STATE 2

v_{O_2} and E are given in arbitrary units. Chlorella, 200 μg chlorophyll per ml, 20°, air.

Expt. No.	*State 1*		*State 2*	
	v_{O_2}	E	v_{O_2}	E
1	110	27.5	113.5	27.5
2	74.5	17.5	73	17
3	72	17	74	17–17.5
4	71	16.5	71.5	17

BONAVENTURA AND MYERS: v (705 nm) in State 2 seems to be smaller than v (705 nm) in State 1 on their recordings in ref. 1. We repeated with a modulated rate electrode the BONAVENTURA AND MYERS[1] experiments. Using their conditions we obtained the same recording as that which they published (Fig. 11 of ref. 1). These authors interpreted the induction stage when a 705-nm continuous light was superimposed on to a 650-nm modulated light as representing the transition from the initial State 2 to a final State 1 stationary level of O_2 evolution. But, if we give a 705-nm modulated light after darkness or after a 650-nm illumination we obtain induction curves which both are nearly similar to the one shown on the above mentioned figure. Therefore, in State 2 (650-nm preillumination) or State 1 (period of darkness) the kinetics of O_2 evolution at 705 nm are not changed and the induction stage corresponds to other phenomena.

We must conclude from Fig. 1 that the correlated changes in rate and in System II active centers E are not caused by a change in the proportion of photons distributed between the two systems.

In order to illustrate the inconsistencies between experiment and the hypothesis of a variation of α, from a different point of view, let us suppose that the observed changes in rate are caused by a variation of α. In Fig. 2 are represented the relationships between the term

$$u(1) = e(1)/(1 - p + e(1)p) = v/v_{max}$$

and the distribution of photons $(1-\alpha)/\alpha$ with various apparent equilibrium constant between the two photoreactions[2] (the 1 and 2 in parentheses refer to the values for States 1 and 2, respectively). The curve for $K = 6$ (on Fig. 2) has been drawn with data taken from O_2 quantum yields[4] and Emerson enhancement[2] measurements in State 1. According to BONAVENTURA AND MYERS, K does not vary. We have experimentally found at 650 nm, during the transition from State 1 to 2 a considerable increase of rate v/v_{max}, passing from 0.74 to 0.94. On curve $K = 6$ of Fig. 2, one sees that this variation of rate can be caused by α passing from 0.51 in State 1 to 0.33 in State 2; this represents a relative variation of α(650 nm) of 35 %. At 680 nm the transition from State 1 to 2 induces a variation of u from 0.79 in State 1 to $u = 0.98$ in State 2. This variation would correspond to a relative variation of α of 66 %; α(680 nm) passing from 0.49 to 0.15*. These changes in rate of O_2 evolution taken in the preceding examples are much more pronounced than those obtained by BONAVENTURA AND MYERS (the light energy of the preillumination at 650 nm was equal to 5000 ergs·cm^{-2}·sec^{-1} in our case).

For such large variations of α we expect first that these variations would be observed on Fig. 1 and second, variations in the absorption spectrum of Chlorella would possibly be found, because a so large shift of pigments could not occur without changes in the apportionment of absorbed light energy to different pigments of perhaps also changes in chemical bondings. In low-temperature (liquid-N_2) absorption spectra measurements of Chlorella illuminated at 650 nm in similar conditions as the preceding experiments, no significant changes of the spectrum could be observed.

Therefore, as demonstrated by Fig. 1, $k_{II}\alpha$ in State 2 stays constant compared

* These variations would be even larger because if α changes, $v_{State\ 2}/v_{max}$ would actually be equal to $u \cdot (\alpha(2)/\alpha(1))$; ($\alpha(2)$ being the value of α in State 2 $< \alpha(1)$).

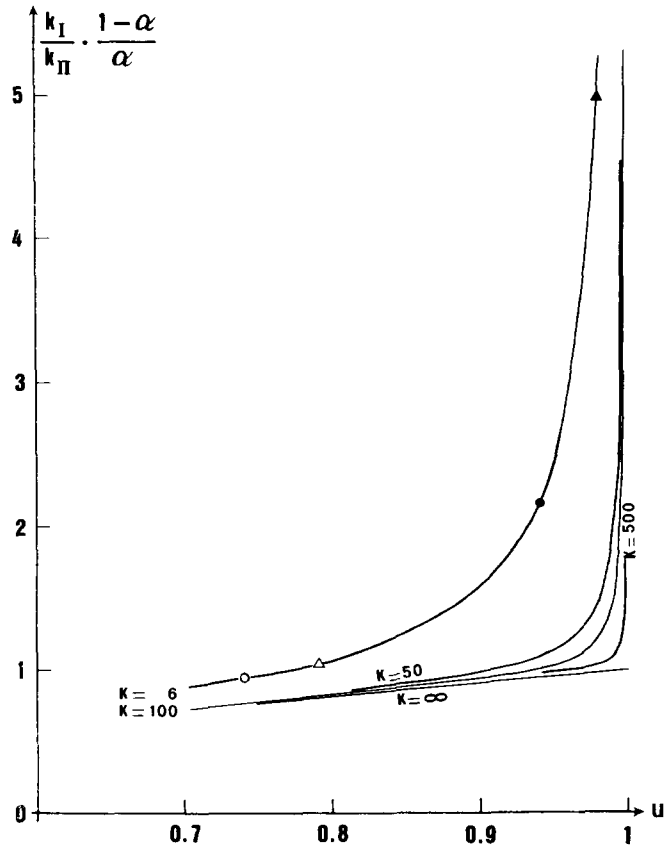


Fig. 2. Curves $(k_I/k_{II}) \cdot (1-\alpha)/\alpha$ as a function of $u = e/(1-p+ep) = v/v_{max}$ for different values of the apparent equilibrium constant K (k_I and k_{II}, quantum yields of Photoreaction I and II, respectively, are considered equal). See ref. 2 for details. Chlorella, 200 μg chlorophyll per ml, 20°, air.

to its value in State 1 and one of possible remaining parameters likely to explain these increases of rate can be the apparent equilibrium constant K between the photoreactions I and II. Fig. 3 represents theoretical curves of steady-state quantum yields as a function of u ($\simeq e$) for different values of apparent K (see Eqn. 7 in ref. 4). The experimental steady-state quantum yield values at 650, 680, 690 and 705 nm in State 2 are found on theoretical curves corresponding to $K = 50$ for a preillumination light 650 nm at 2500 ergs·cm^{-2}·sec^{-1} during 10 min, and K between 100 and 500 for the same preillumination but at 5000 ergs·cm^{-2}·sec^{-1}. For State 1, the data lie on the $K = 6$ curve. So Light 2 increase the apparent equilibrium constant between the two photosystems. We can notice on Fig. 3 that if K stays equal to 6 in State 2, no matter how α varies, the quantum yield Φ_S would not increase. As seen on the curve corresponding to $K = 6$ when $u \simeq e\uparrow$ and $\alpha\downarrow$, Φ_S stays constant (middle point of the curve) or decreases. When algae pass from State 1 to State 2 there is actually an increase of the O_2 quantum yield, as shown in the present work or by other investigators. Therefore Light 2 increase the rate of electron transfer from Photosystem II to

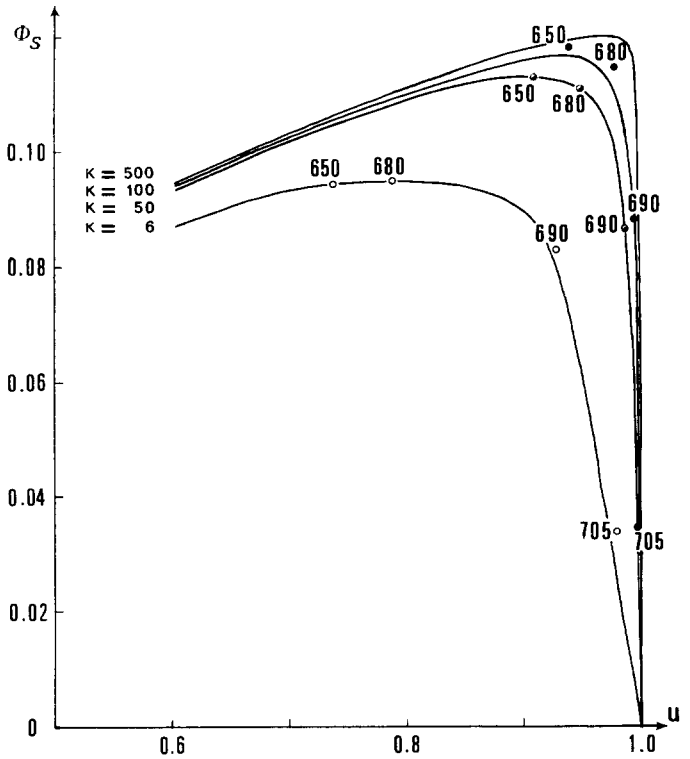


Fig. 3. Steady-state quantum yields (Φ_S) at different wavelengths as a function of $u = e/(1 - p + e/p) = v/v_{max}$ in State 1 and in State 2; ○, data obtained in State 1 ($K = 6$); ◒ ●, data in State 2 ($K = 50$ and K between 100 and 500). Chlorella, 200 μg chlorophyll per ml, 20°, air.

Photosystem I; it does not change the quantum yield of centers or the pigment distribution between the two photosystems.

The following remarks can be made at this time:

(1) an other argument against the variation of α in the Bonaventura–Myers effect is given by the action of 3-(3,4-dichlorophenyl)-1,1-dimethylurea on algae. MURATA[5] showed that the fluorescence spectrum of *Porphyridium cruentum* at low temperature, when the algae are preilluminated with a System II light, does not vary as compared to a System I preillumination, if 3-(3,4-dichlorophenyl)-1,1-dimethylurea is added. With 3-(3,4-dichlorophenyl)-1,1-dimethylurea (high concentration), the State 2 was never observed because 3-(3,4-dichlorophenyl)-1,1-dimethylurea is an inhibitor of Photosystem II.

The Bonaventura–Myers effect and the effect of salt addition to algae exhibit different behaviour in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Increase of the ionic strength, by KCl, for instance, decreases the fluorescence level even in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea[6,7]; and moreover, with salt addition a noticeable variation in the absorption spectrum was reported[7]. So, in this latter case, perhaps variation of factor α occurs (this may be also the case in strong light, since a spectral change was also observed)[7].

(2) A long 680-nm illumination can also induce an increase of e and v/v_{max} for

the studied wavelengths (650, 680 and 690 nm), but the effect is much weaker than with long 650-nm preillumination (see in Fig. 1 the observed changes for 680 nm during a 680-nm preillumination).

(3) The equilibrium constant between E (or Q) and A does not vary during the transition from State 1 to 2. After a S_{II} illumination a concomitant increase of A (measured by the burst of O_2) and E (measured by the microburst of O_2) is observed. We found a value of the equilibrium constant nearly equal to 1 like in State 1 (ref. 3).

(4) The effect of a long Light 2 illumination can explain why some authors[8] have found little Emerson enhancement with algae. As a matter of fact, in State 2 there may be nearly no Emerson enhancement. The Emerson effect is caused by the large difference in the steady state concentrations of E at 705 and 650 nm, so the intermediate value of E obtained with 2 lights is not the average of the 2 values but is higher. In State 2, the concentrations of E at 650 and 705 nm are nearly equal; it is the reason why there is little Emerson enhancement.

(5) The kinetics of the changes in O_2 evolution observed during the transition from State 1 to State 2 seems to depend only on the product $i \cdot t$ (intensity·time). The higher is the preillumination 650-nm light intensity, the shorter is the time necessary to obtain the same increase of the rate (or of E). However, we have only studied the Bonaventura–Myers effect within a limited range of light intensities (2500–5000 ergs·cm^{-2}·sec^{-1}): our remark therefore is subject to caution and requires a verification for very different light intensities.

It seems that under the effect of Light 2, a "product" is slowly formed which modifies the electron transfer between the two photoreactions; this product would be "destroyed" by Light 1 or with less efficiency by darkness. On account of the relatively slowness of the changes, we can suppose with VREDENBERG[9] that the ion changes across the chloroplast membranes should play a part in the explanation of this phenomenon.

REFERENCES

1 C. BONAVENTURA AND J. MYERS, *Biochim. Biophys. Acta*, 189 (1969) 366.
2 M.-J. DELRIEU AND Y. DE KOUCHKOVSKY, *Biochim. Biophys. Acta*, 226 (1971) 409.
3 A. JOLIOT, *Physiol. Vég.*, 6 (1968) 235.
4 M-J. DELRIEU, in H. METZNER, *Progress in Photosynthesis Research*, Vol. 2, Tübingen, 1969, p. 110.
5 N. MURATA, *Biochim. Biophys. Acta*, 172 (1969) 242.
6 Y. DE KOUCHKOVSKY, in J. G. GREGORY, *Abstr. 8th Int. Congr. Biochem.*, Staples Printer, Rochester (U.K.), 1970, p. 139.
7 Y. DE KOUCHKOVSKY, presented at the *2nd Int. Congr. Photosynthesis Res., Stresa, Italy, June 1971*, in the press.
8 T. PUNNETT, *Science*, 171 (1971) 284.
9 W. J. VREDENBERG, *Biochim. Biophys. Acta*, 223 (1970) 230.