Biochimica et Biophysica Acta, 546 (1979) 256—269

BBA 47650

A BACKFLOW OF ELECTRONS AROUND PHOTOSYSTEM II IN *CHLORELLA* CELLS

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(Received August 18th, 1978)

Key words: Photosystem II; Oxygen evolution; Electron transport; Photosynthesis (Chlorella)

Summary

A study was made with a modulated oxygen electrode of the effect of variations of oxygen concentration on photosynthetic oxygen evolution from algal cells. When *Chlorella vulgaris* is examined with a modulated 650 nm light at 22°C, both the oxygen yield and the phase lag between the modulated oxygen signal and the light modulations have virtually constant values between 800 and 120 ergs · cm^{-1} · s^{-1} if the bathing medium is in equilibrium with the air. Similar results are obtained at 32°C between 1600 and 120 ergs · cm^{-2} · s^{-1}. Under anaerobic conditions both the oxygen yield and the phase lag decrease if the light intensity is lowered below about 500 ergs · cm^{-2} · s^{-1} at 22°C or about 1000 ergs · cm^{-2} · s^{-1} at 32°C. A modulated 706 nm beam also gives rise to these phenomena but only at significantly lower rates of oxygen evolution. The cells of *Anacystis nidulans* and *Porphyridium cruentum* appear to react in the same way to anaerobic conditions as *C. vulgaris*. An examination of possible mechanisms to explain these results was performed using a computer simulation of photosynthetic electron transport. The simulation suggests that a backflow of electrons from a redox pool between the Photosystems to the rate-limiting reaction between Photosystem II and the water-splitting act can cause a decrease in oxygen yield and phase lag. If the pool between the Photosystems is in a very reduced state a significant cyclic flow is expected, whereas if the pool is largely oxidized little or no cyclic flow should occur. It is shown that the effects of 706 nm illumination and removal of oxygen can be interpreted in accordance with these proposals. Since a partial inhibition of oxygen evolution by 3-(3.4-dichlorophenyl)-1,1-dimethylurea (10^{-8} M) magnifies the decreases

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

in oxygen yield and phase lag, it is proposed that the pool which cycles back electrons is in front of the site of 3-(3,4-dichlorophenyl)-1,1-dimethylurea inhibition and is probably the initial electron acceptor pool after Photosystem II.

Introduction

In the operation of the modulated oxygen electrode a layer of *Chlorella* cells (or isolated chloroplasts) lying on a shiny platinum electrode is illuminated by a light beam whose intensity is periodically modulated. The cells respond to this illumination by producing waves of oxygen which interact with the platinum electrode and cause an alternating current to flow in the measuring circuit. The mathematical analysis of the operation of this electrode was performed by Joliot et al. [1] who showed that both the diffusion of oxygen from the cells to the platinum electron and the rate-limiting chemical reaction between Photosystem II and the watersplitting act had a profound influence on the observed signal. Joliot et al. [1] observed that the phase of the signal was independent of the intensity of the modulated light beam and concluded that the rate-limiting reaction must obey first-order kinetics. The phase independence of the light intensity has been confirmed in this laboratory many times both for *Chlorella* and isolated spinach chloroplasts. However, we have now discovered that if *Chlorella* cells are placed in an anaerobic situation the signal phase lag does show a marked dependence on the light intensity. It is the purpose of this paper to illustrate this phase dependence on light intensity under various experimental conditions and present the results of a computer simulation of the operation of Photosystem II which offers an explanation of these observations.

Methods

This study was performed on three different algae, namely *Chlorella vulgaris*, *Anacystis nidulans* and *Porphyridium cruentum*. The apparatus used with *Chlorella* including the oxygen electrode, associated electrical circuit, vector voltmeter, light chopper and optical set-up has already been described [2]. The apparatus used with *Anacystis* and *Porphyridium* was kindly made available by Dr. J. Barber, Botany Department, Imperial College of Science and Technology, London, England. The design and mode of use of this oxygen electrode were essentially identical to the one described above.

Chlorella was cultured as described by Sinclair and Arnason [3] while *Anacystis* and *Porphyridium* were grown in continuous aeration and illumination at 20°C in media given in Kratz and Myers [4] and Jones et al. [5], respectively. The bathing medium for *Chlorella* contained 20 mM NaCl and 10 mM potassium phosphate buffer, pH 7.6, which was identical to that used with *Anacystis* except that the pH was altered to 6.9. *Porphyridium* was examined in a medium containing 0.5 M NaCl and 20 mM Tris buffer, pH 7.7. The medium bathing the experimental material was made aerobic or anaerobic by bubbling suitable gas mixtures through the solution reservoirs so that a change in condi-

tions could be made by switching from one reservoir to another. For an aerobic solution the gas mixture was either air alone of 90% air/10% CO_2 and for an anaerobic solution, the reservoir was bubbled with pure nitrogen or 90% N_2/ 10% CO_2 or 90% N_2 containing 1000 ppm O_2 plus 10% CO_2.

The experimental material was exposed to an anaerobic solution initially and illuminated with monochromatic, modulated light. Once the rate of oxygen evolution, as measured by the amplitude of the modulated signal, and the phase lag between the oxygen signal and the light intensity modulations had reached steady values, the light intensity was varied and the resultant amplitude and phase lag changes were measured. The light intensity was altered either by varying the modulated light intensity or by adding to the modulated light a non-modulated light of the same wavelength. In the latter case, changes in the amplitude of the oxygen signal measured changes in the oxygen yield caused by varying the light intensity. These measurements were repeated under anaerobic conditions and then under aerobic conditions once more to check the original observations. Most of these experiments were performed at room temperature ($21 \pm 1°C$) but some were done at 32°C. Observations were also made of the dependence of the phase lag of *Chlorella* cells on the modulation frequency under anaerobic conditions at different light intensities of modulated light. The results were fitted to the equations derived by Joliot et al. [1] by means of an iterative curve-fitting procedure. In some experiments the effect of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) on oxygen evolution from *Chlorella* was examined in the presence and absence of oxygen. It was added, dissolved in ethanol, to produce a final concentration of 10^{-8} M. Equivalent additions of ethanol without DCMU had no effect. The modulation frequency used in these experiments was 20 Hz unless stated otherwise.

Results

A modulated 650 nm light beam whose intensity was 120 ergs $\cdot$ cm^{-2} $\cdot$ s^{-1} was shone upon a monolayer of *Chlorella* cells in the presence of different intensities of unmodulated light of the same wavelength. The modulated signal size and its phase were measured at the different light intensities under both aerobic and anaerobic conditions and the results obtained at 22°C are shown in Fig. 1 and those obtained at 32°C are shown in Fig. 2. At both temperatures the oxygen yield, which is proportional to the signal size, remained virtually constant when measured under aerobic conditions but displayed a marked dependence on the light intensity when measured anaerobically. At both temperatures the oxygen yield at high light intensities was greater under anaerobic conditions than under aerobic conditions but as the intensity was lowered this was reversed. It is apparent that the diminution in the oxygen yield began at much higher light intensities when observations were made at 32°C rather than at 22°C. The phase lag of the oxygen signal did not vary significantly with the light intensity when the experiment was performed aerobically but it did decrease in value as the light intensity was lowered under anaerobic conditions. The influence of raising the temperature was to raise the value of light intensity below which the phase lage became sensitive to the light intensity. The intensity below which the phase lag and oxygen yield decreased varied quite

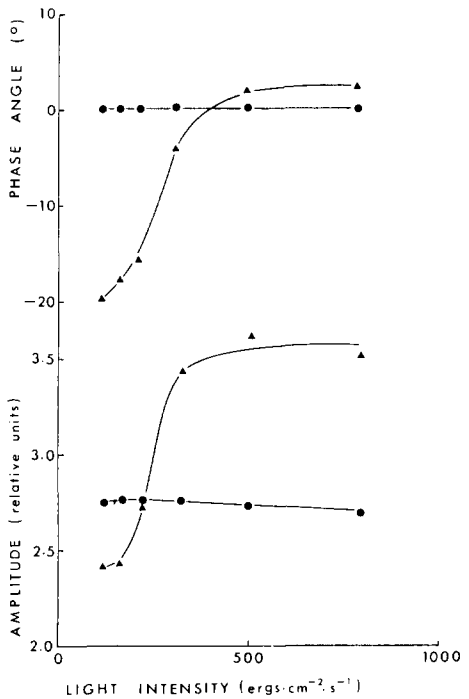


Fig. 1. The phase angle (ϕ) and amplitude (A) measured under aerobic (●) and anaerobic (▲) conditions for a monolayer of *C. vulgaris*. The cells were illuminated with a modulated 650 nm light of mean intensity 120 ergs · cm^{-2} · s^{-1} to which were added different non-modulated lights of the same wavelength. The total intensity is plotted on the abcissa. The amplitude of the modulated signal is proportional to the oxygen yield at each intensity. The phase angle is arbitrarily taken as zero for the aerobic conditions at an intensity of 780 ergs · cm^{-2} · s^{-1} and a negative value implies a decrease in phase lag of the oxygen modulations behind the light fluctuations. The bathing medium was equilibrated with a gas mixture containing 90% air and 10% CO_2 for aerobic conditions with a mixture of 90% N_2 plus 1000 ppm oxygen and 10% CO_2 for anaerobic conditions. The temperature was 22°C.

markedly between different experiments, ranging from 1000 to 300 ergs · cm^{-2} · s^{-1} at room temperature. The cause of this variation is not known. In part it may have been due to changes in *Chlorella* itself but leakage of oxygen into the perfusion medium may also have been a factor.

If the oxygen yield and phase lag were measured at higher light intensities than those used above it was found that the phase lag was no longer dependent on the presence or absence of oxygen but a new phenomenon appeared. Between 1000 and 10 000 ergs · cm^{-2} · s^{-1} the phase lag gradually decreased by about 10° and this was accompanied by a decrease in oxygen yield of about 40%. It might be suspected that the decrease in oxygen yield was due to CO_2 fixation process rate-limiting the photosynthesis but this would not explain the decrease in phase lag. In the experiment shown in Fig. 3, *Chlorella* cells were examined in a medium which had been bubbled with pure nitrogen. The rate of oxygen evolution and the changes in phase lag were measured at different light intensities, then 2 mM $NaHCO_3$ was added to the medium and the same observations were made. While lack of bicarbonate severely restricted the rate of oxygen evolution, it had no discernable effect on the phase lag. Thus it is

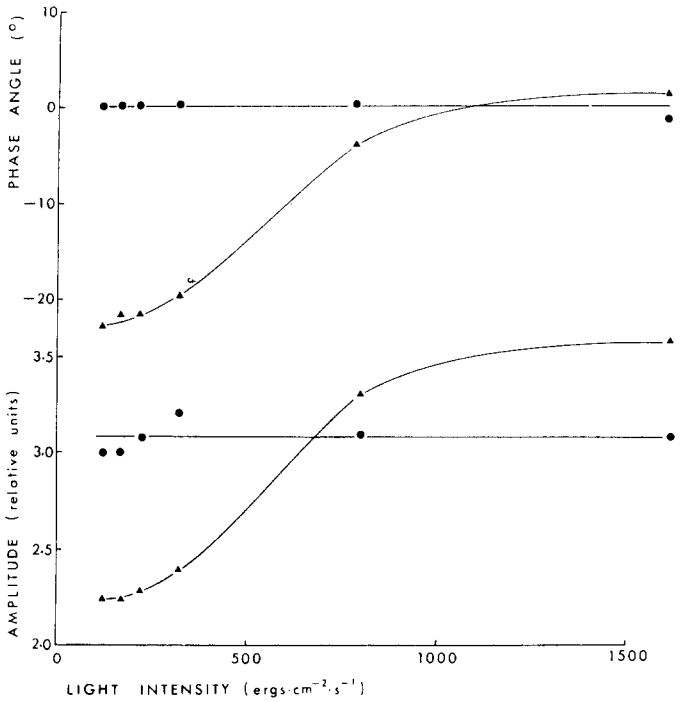


Fig. 2. The phase angle (ϕ) and amplitude (A) measured under aerobic (●) and anaerobic (▲) conditions for a monolayer of *C. vulgaris*. The temperature was 32°C. Other conditions as for Fig. 1.

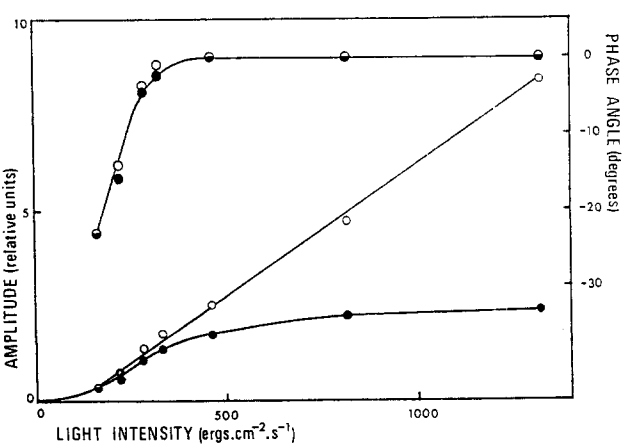


Fig. 3. The effect of the addition of 4 mM $NaHCO_3$ on the amplitude and phase angle measured under anaerobic conditions. A monolayer of *C. vulgaris* was illuminated with different intensities of modulated 650 nm light and the amplitude and phase angle were measured before and after the addition of $NaHCO_3$ to a solution which had been equilibrated with nitrogen. The temperature was 20°C. The open circles represent results obtained in the presence of 4 mM $NaHCO_3$ and the closed circles those obtained in its absence. The large symbols are the phase angle measurements and the small ones the amplitude values. The phase angle was arbitrarily taken as zero at the highest intensity in the presence of HCO_3^- and negative values represent a decrease in phase lag.

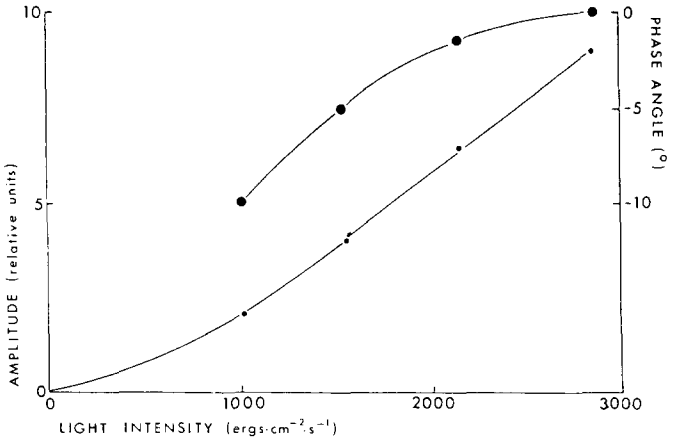


Fig. 4. The amplitude and phase angle as a function of the intensity of a modulated 706 nm light for a monolayer of *Chlorella* measured under anaerobic conditions. The temperature was 20°C. Zero phase angle taken as the phase measured at 2850 ergs · cm^{-2} · s^{-1} and negative values represent decreasing phase lags.

doubtful that limitations of CO_2 fixation can explain the results obtained at high light intensities.

The preceding experiments revealed that under anaerobic conditions the phase lag and oxygen yield of the oxygen signal from *Chlorella* were both smaller at low intensities of 650 nm light than at high intensities. As is shown in Fig. 4 this was also true when 706 nm light was used to illuminate *Chlorella* cells, although the intensities used were much higher due to the poor absorption of the light of this wavelength. However, there was an important difference between the results obtained with 650 and 706 nm light and this is illustrated in Fig. 5. A layer of *Chlorella* was first exposed to different intensities of modulated 650 nm light and then to different intensities of modulated 706 nm light under anaerobic conditions. The rates of oxygen evolution and phase lags were measured and in Fig. 5 the decreases in phase lag for the two wavelengths are plotted against the corresponding rates of oxygen evolution. It is apparent that a given phase lag decrease occurs at a lower rate of oxygen evolution in 706 nm light as compared with 650 nm light. From this it can be concluded that illumination with 706 nm light alters conditions within the *Chlorella* cell from those existing during illumination with 650 nm light and these changed conditions discourage the appearance of the phase dependence on light intensity.

Joliot et al. [1] developed mathematical equations which describe the operation of the modulated oxygen electrode assuming that the reaction with rate-limits oxygen evolution between Photosystem II and the water-splitting act obeys first-order kinetics. While these equations have been shown to give an excellent description of the results obtained under aerobic conditions for both *Chlorella* [3] and isolated spinach chloroplasts [2], they do not predict the results obtained here under anaerobic conditions. Despite its doubtful validity, the mathematical theory of Joliot et al. [1] was applied to the phase lag values obtained at different modulation frequencies under anaerobic conditions for

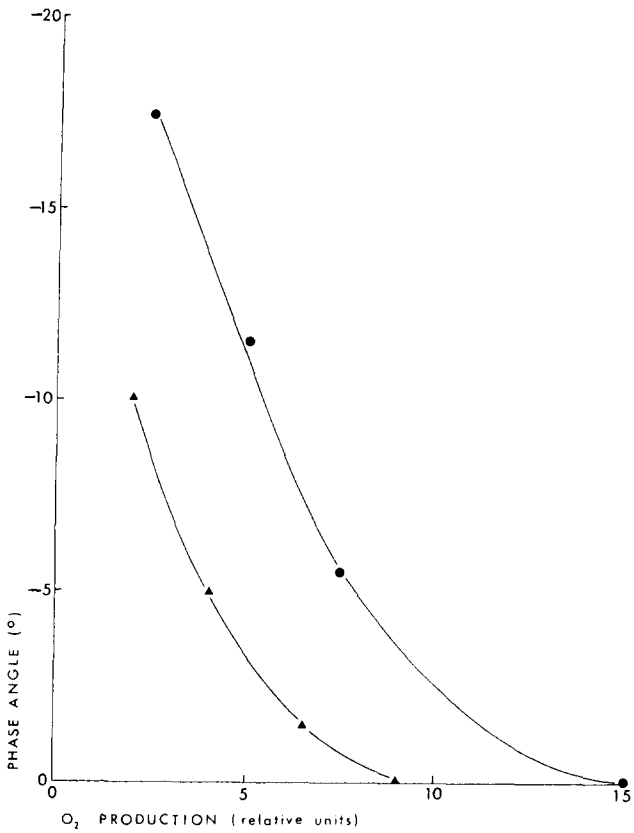


Fig. 5. The phase angle as a function of the relative rate of oxygen production for a monolayer of *C. vulgaris* illuminated with different intensities of modulated 650 nm light (●) and of modulated 705 nm light (▲) under anaerobic conditions. The temperature was 20°C. the phase angles measured at the highest rates of oxygen evolution int he two lights were equal and were taken as zero. Negative phase angles imply a decrease in phase lag.

TABLE I

CALCULATED VALUES OF THE APPARENT RATE CONSTANT FOR THE RATE-LIMITING REACTION OF OXYGEN EVOLUTION AS A FUNCTION OF LIGHT INTENSITY

The phase lag values observed at a series of modulation frequencies for a monolayer of *C. vulgaris* under anaerobic conditions were described with the phase equation from the theoretical treatment by Joliot et al. [1]. The best fitting values of the parameters in the phase equation are shown below. Temperature = 19°C.

Light intensity (ergs · cm^{-2} · s^{-1})	Phase intensity at zero frequency (°)	Distance of closest O_2 sources from Pt electrode (μm)	Apparent rate constant (s^{-1})	Standard deviation (°)
1820	0	3.5	176	0.6
130	0	3.5	249	0.9
48	0	3.8	369	2.0

three different light intensities. The parameters which gave the best fit of theory to experimental results are shown in Table I.

An experiment was performed in which *Chlorella* cells were exposed to 10^{-8} M DCMU in the presence and absence of oxygen at a modulated 650 nm light intensity of 800 ergs · cm^{-2} · s^{-1} and at room temperature. In the presence of oxygen, DCMU decreased the rate of oxygen evolution by about 10% and had no effect on the phase of the signal. In the absence of oxygen, DCMU decreased the rate of oxygen evolution by 50% and decreased the phase lag by 18°. In the absence of DCMU the rate of oxygen evolution was unchanged whether oxygen was present or not but the phase lag was 10° smaller in the absence of oxygen.

The results of measurements made at a series of modulated 650 nm light intensities on *A. nidulans* are shown in Fig. 6. Under aerobic conditions the rate of oxygen evolution was a linear function of the light intensity while the phase lag remained constant. Removal of oxygen resulted in a non-linearity in

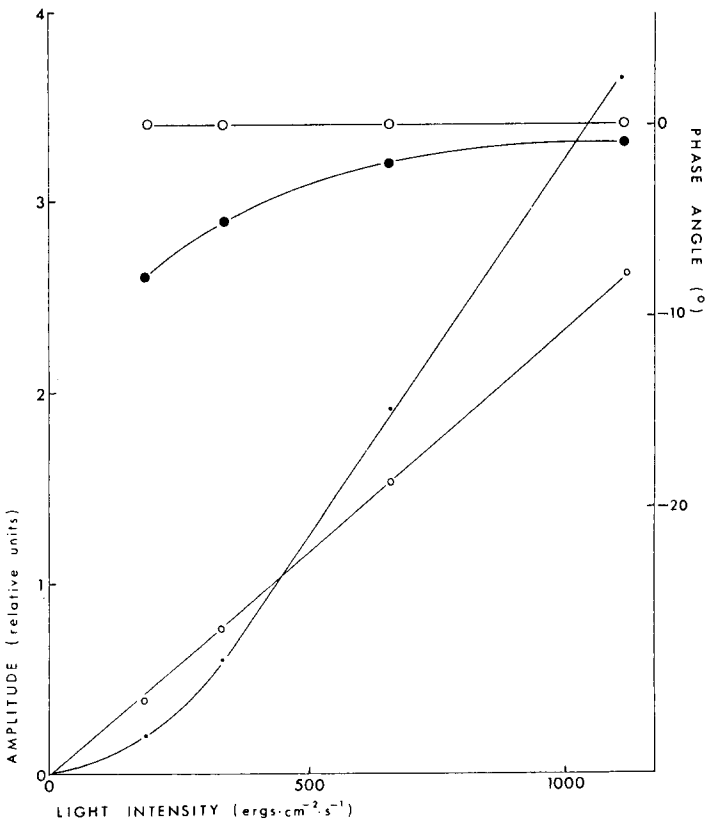


Fig. 6. The influence of oxygen concentration on the amplitude and phase agle for a 7 μm layer of *A. nidulans*. The open symbols represent results obtained under aerobic conditions and the closed symbols those obtained under anaerobic conditions. The small symbols represent the amplitude results which are proportional to the rate of oxygen evolution. The large symbols are the phase angles where the zero value is taken as the phase angle at the highest intensity under aerobic conditions and negative values correspond to a decrease in phase lag. The temperature was 21°C.

the rate of oxygen evolution versus intensity results with the oxygen yield at low intensities being less than that in the presence of oxygen. It is also apparent that the phase lag decreased with light intensity when oxygen was absent. Jones and Myers [6] also noted as non-linearity in their rate of oxygen evolution versus light intensity plots obtained with *A. nidulans* in the absence of oxygen.

A brief study was also performed on *P. cruentum* which yielded results similar to those for the other two algal types, i.e., both the phase lag and the oxygen yield decreased when oxygen was removed from the bathing medium at low intensities of 544 nm modulated light.

Discussion

It has been shown that the phase lag and oxygen yield of the oxygen signal from algal cells decrease with light intensity under anaerobic conditions. These observations are in conflict with the first-order model of Joliot et al. [1] which predicts that both of these parameters should be independent of the light intensity. An attempt was made to reconcile the Joliot model to these results by assuming that the enzyme which catalysed the first-order rate-limiting reaction was labile under the conditions of these experiments and gave rise to a different rate constant at each light intensity. However, a decrease in phase lag requires an increase in the rate constant which is incompatible with the need to decrease the rate constant to produce a smaller oxygen yield so this attempt was abandonned. The second possible explanation which was examined was that under anaerobic conditions at low light intensities a new reaction, which obeyed second-order kinetics, became rate-limiting. The differential equations describing such a model were solved numerically with the aid of a computer and the solutions revealed that the phase lag would increase as the light intensity is lowered. Thus a second-order model fails to explain the experimental findings.

The hypothesis which has proved most useful in understanding these results is that at low light intensities under anaerobic conditions there is a backflow of electrons which interferes with oxygen production and gives rise to the observed phenomenon. It is illustrated first in a simplified form in the following differential equations:

$$\frac{d[O_2]}{dt} = k[X^+] \tag{1}$$

$$\frac{d[X^+]}{dt} = k_c[X]I_0(1 - \sin \omega t) - k[X^+] - k_L[L][X^+] \tag{2}$$

where Eqn. 1 expresses the dependence of the rate of O_2 evolution on the concentration of the oxidized substance $[X^+]$ and Eqn. 2 describes the production of X^+ as a light driven process. I_0 is the maximum intensity of the light absorbed by Photosystem II which is modulated at an angular frequency, ω. [X] is the concentration of X in the reduced state, k_c, k, and k_L are rate coefficients and [L] is the concentration of the reduced form of some substance L which can reduce X^+. Thus the last term in Eqn. 2 describes the backflow of

electrons postulated above. If [L] is reduced to zero, these equations become identical to those used by Joliot et al. [1] when they set up their first-order model.

The steady-state solution to these equations includes a modulated production of oxygen. The amplitude and phase lag of this modulation are given below:

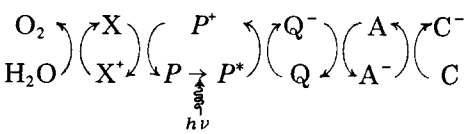
$$\text{Phase lag} = \tan^{-1}\left(\frac{\omega}{k + k_L[L]}\right) \tag{3}$$

$$\text{Amplitude} = \frac{k_c k[X] I_0}{\sqrt{\omega^2 + (k + k_L[L])^2}} \tag{4}$$

where it has been assumed that [L] is a constant in the steady-state and that the intensity is sufficiently low so that most of the substance X is in the reduced form. It is apparent that the phase lag and amplitude (and hence the oxygen yield) depend not only on the value of k as in the Joliot model but also on the product $k_L(L)$. An increase in the value of $k_L(L)$ will decrease both of these parameters while a decrease in $k_L(L)$ will cause an increase so giving rise to the sort of changes which were observed.

The phase lag Eqn. 3 is identical in form to that obtained by Joliot et al. [1] with their original model except that k in their equation has been replaced by $(k + k_L(L))$. Thus the curve-fitting procedure which was applied to the phase values at three different light intensities under anaerobic conditions remains valid (see Table I). If it is assumed that at the highest intensity used in that experiment (L) = 0 and at the lowest intensity that (L) had its maximum value the difference in the apparent rate constants gives an estimate for the magnitude of $k_L(L)_{max}$, namely 193 s^{-1}. It should be noted that if either of the above assumptions is incorrect this estimate will be less than the true value.

Since the validity of these promising solutions was limited by the assumptions underlying the equations, i.e., the constancy of (L), the light intensity being sufficiently low and the modulations of $[X^+]$ having the same phase as the light, it was decided to set up a more complex set of equations which did not require these assumptions. With this complex set of equations it was hoped to investigate why these effects appeared more readily in 650 than in 706 nm light, why the absence of oxygen promoted their appearance, what the most probably identity of L was and why these effects appeared only at low light intensities. The reaction scheme on which the equations were based is shown below:



and the set of equations is as follows:

$$\frac{d[O_2]}{dt} = k[X^+] \tag{5}$$

$$\frac{d[X^+]}{dt} = k_1[P^+][X] - k_{-1}[P][X^+] - k[X^+] \quad (6)$$

$$\frac{d[P^*]}{dt} = k_C I[P] - k_D[P^*] + k_{-2}[Q^-][P^+] - k_2[Q][P^*] \quad (7)$$

$$\frac{d[P^+]}{dt} = k_{-1}[P][X^+] - k_1[P^+][X] + k_2[Q][P^*] - k_{-2}[Q^-][P^+] \quad (8)$$

$$\frac{d[Q^-]}{dt} = k_2[Q][P^*] - k_{-2}[Q^-][P^+] + k_{-3}[A^-][Q] - k_3[A][Q^-] \quad (9)$$

$$\frac{d[A^-]}{dt} = k_3[A][Q^-] - k_{-3}[A^-][Q] - k_4[A^-] \quad (10)$$

$$\frac{d[C^-]}{dt} = k_4[A^-] \quad (11)$$

These equations describe the flow of electrons from water via X to the reaction centre of Photosystem II, *P*, and then through the first and second electron acceptor pools, Q and A, to pool C. Oxidation of the second electron acceptor pool by C represents the removal of electrons from Photosystem II and includes the removal of electrons transported through Photosystem I. Variations in the values of the rate constant k_4 can therefore be used to describe changes in Photosystem I activity or changes in the activity of any entity capable of oxidizing A^-. Apart from the first and last steps, back reactions have been included at every step including a backreaction between Q^- and P^+.

The solutions to these equations which constitute a simulation of the flow of electrons through Photosystem II, were obtained numerically using a Runge-Kutta-Gill method. The equations were normalized so that the concentrations of the various redox compounds could vary between 0 and 1 and it was assumed that when the light was switched on, X and *P* were in a reduced form while Q, A and C were oxidized. These equations were solved for values of $k_C I$ varying over several orders of magnitude and for

$k = 170—1000\ s^{-1}$

$k_1 = 200—2000\ s^{-1}$

$k_{-1} = 200—1000\ s^{-1}$

$k_2 = 10^6—10^8\ s^{-1}$

$k_{-2} = 10^3—10^5\ s^{-1}$

$k_3 = 100—1000\ s^{-1}$

$k_{-3} = 100—1000\ s^{-1}$

$k_4 = 0—1000\ s^{-1}$

Various sets of values of the rate constants within these ranges were composed and used for calculation. The results of the simulation for one such

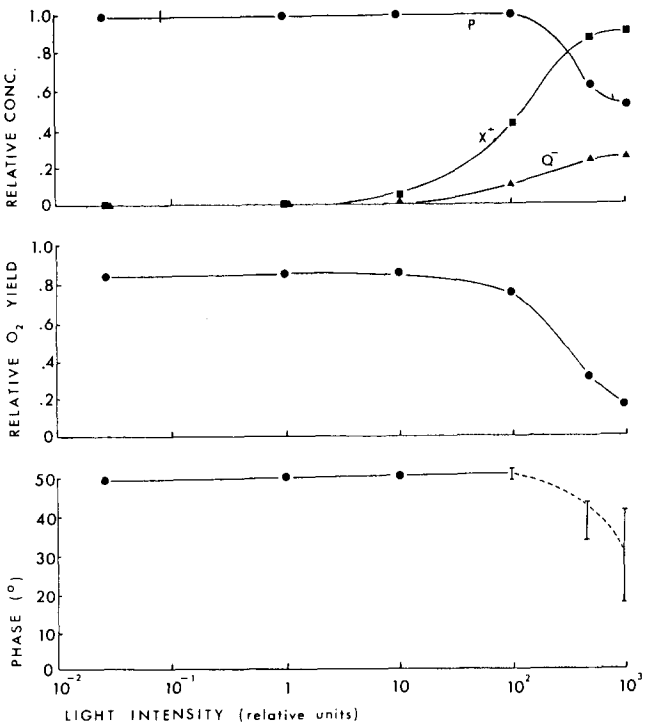


Fig. 7. The results of a computer simulation of Photosystem II based on Eqns. 5—11. The rate constants were given the following values; $k = 170\ s^{-1}$, $k_1 = 2000\ s^{-1}$, $k_{-1} = 200\ s^{-1}$, $k_D = 1000\ s^{-1}$, $k_2 = 10^6\ s^{-1}$, $k_{-2} = 1000\ s^{-1}$ and $k_3 = 1000\ s^{-1}$. The concentrations and oxygen yield were normalized to a maximum value of unity.

set of rate constants are shown in Fig. 7 and they exhibit two characteristics which were consistently observed. Firstly, at high intensities both the oxygen yield and the phase lag decreased in value although the phase lag was difficult to evaluate due to distortions of the waveform. This resembles the experimental results at high light intensities obtained both in the presence and absence of oxygen. As can be seen from the upper graph these decreases occur where $[X^+]$ attains significant values and so the solutions given in Eqns. 3 and 4

TABLE II

RESULTS OF COMPUTER SIMULATION OF PHOTOSYSTEM II INVOLVING AN INTERACTION BETWEEN A^- AND X^+

The values given below were calculated in a simulation based on Eqns. 5, 6A, 7, 8, 9, 10A, and 11. The relative intensity was 100 and $k_A = 500\ s^{-1}$. The other rate constants were as given in Fig. 7. $[Q^-]$ and $[A^-]$ are normalized concentrations.

k_4 (s^{-1})	$[Q^-]$	$[A^-]$	Phase lag (°)	Oxygen yield
20	0.68	0.62	16.9	0.34
100	0.41	0.33	24.8	0.45
500	0.21	0.13	40.5	0.59
1000	0.16	0.07	47.3	0.64

would not be valid. Secondly, at low light intensities the simulation predicts that the oxygen yield and phase lag will have large constant values. This describes the results for experiments performed in the presence of oxygen but not those for experiments performed in the absence of oxygen. Thus the identification of the reaction centre P with the subtance L fails to account for the low values of oxygen yield and phase lag found under anaerobic conditions.

A significant change occurred in the computer simulation when a redox pool between the two Photosystems was identified with L of Eqn. 2. Eqns. 6 and 10 were replaced by Eqns. 6A and 10A when A was chosen as the redox pool:

$$\frac{d[X^+]}{dt} = k_1[P^+][X] - k_{-1}[P][X^+] - k[X^+] - k_A[A^-][X^+] \quad (6A)$$

$$\frac{d[A^-]}{dt} = k_3[A][Q^-] - k_{-3}[A^-][Q] - k_A[A^-][X^+] - k_4[A^-] \quad (10A)$$

As shown in Table II and unlike the results of the previous simulation, the phase lag and the oxygen yield are very sensitive to the value of the rate constant, k_4. When k_4 is large in comparison with k_A, the Q and A pools are almost entirely oxidized and the phase lag and oxygen yield have large values. But when k_4 is small in comparison with k_A, the Q and A pools are largely reduced and the phase lag and oxygen yield have small values. Hence the cyclic flow of electrons from A^- to X^+ depends on the intersystem pool being largely reduced which in turn depends on how rapidly electrons leave this pool for Photosystem I or any suitable electron acceptor and on this basis some of the experimental results become explicable. For example, it was shown in Fig. 5 that in a 706 nm light the phase lag at a given rate of oxygen evolution was larger than that in 650 nm light. But since a greater proportion of 706 nm rather than 650 nm light will be absorbed by Photosystem I, the intersystem pool A will be more oxidized in the 706 nm light and so a larger phase lag would be expected. Also the need for anaerobic conditions to be observed can be related to the work of Diner and Mauzerall [7] and Schreiber and Vidaver [8]. These workers proposed that removal of oxygen would cause A to bcome more reduced, which in the presence context would give rise to the observed decreases of phase lag and oxygen yield. Support for the idea that the absence of oxygen results in A becoming more reduced which then causes a cyclic flow to X^+ comes from a study by Diner [9]. This author demonstrated that the rate of deactivation of the S_2 and S_3 states in the dark was significantly speeded up by making the bathing medium anaerobic. Since deactivation implies a reduction, these results are consistent with the proposed interaction between A^- and X^+.

But these simulation results are not unique to an interaction between A^- and X^+. A further simulation was performed in which Q^- and X^+ were allowed to interact and it was found that varying the rate at which electrons were removed from Q^- gave rise to changes in the oxygen yield and phase lag similar to those described above. Fortunately, the experiment performed with DCMU offers a way of discriminating between the above possibilities. Partial inhibition of the flow of electrons between Photosystem II and Photosystem I by DCMU should result in a redox pool in front of the site of inhibition becoming more

reduced and one behind the site becoming more oxidized. Since DCMU is supposed to operate after Q and before A this should mean Q becomes more reduced and A more oxidized and so if Q^- is the source of the electrons to reduced X^+, DCMU should accentuate the decreases in oxygen yield and phase lag whereas if A^- is the source, DCMU should have the opposite effect. Quite clearly the results with DCMU conform with the first of these possibilities and suggest that Q^- rather than A^- interacts with X^+.

If Q^- does interact with X^+ it appears likely that Q^- can also interact with oxygen since it was shown that the signal and phase lag were larger in DCMU-poisoned algae if oxygen was present. This contrasts with the proposal by Diner and Mauzerall [7] that A^- was oxidized by oxygen but does not necessarily conflict with it. It is possible that both A^- and Q^- interact with oxygen or perhaps A^- does only via Q^- since Bouges-Bocquet et al. [10] have proposed that A^- can reduce Q.

Two aspects of the results remain to be incorporated into the model presented above. The first is the influence of temperature which could be explained if the rate constant determining the cycling back of electrons to X^+ increased more rapidly with temperature than the other rate constants. This would make this pathway of electron transport relatively more important at elevated temperatures as observed. The second aspect is the appearance of the decreases in phase lag and oxygen yield only at low light intensities. There are several hypotheses which could explain this observation and attempts are currently being made to discriminate between them.

One finding obtained in this study has not been discussed, namely the lower oxygen yield obtained in the presence of oxygen for light intensities above 500 ergs $\cdot$ cm^{-2} $\cdot$ s^{-1} at room temperature (see Fig. 1). As this phenomenon was not the main subject of this investigation it is difficult to offer an explanation for it. However, it would not seem unreasonable to suggest that it may reflect the generation of an inhibitory substance due to the reduction of oxygen.

In conclusion it is proposed that there is a redox pool between the two Photosystems but in front of the site of action of DCMU can cycle back electrons to reduce X^+, an oxidized substance involved in the rate-limiting reaction of oxygen evolution. This electron transport pathway can cause a significant decrease in the rate of oxygen evolution when the intersystem redox pool is largely reduced, i.e., when oxygen is absent and the light intensity very low.

Acknowledgments

This work was supported by funds from the National Research Council of Canada, and funds awarded to Dr. J. Barber by the Scientific Research Council of Great Britain.

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