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# A KINETIC ANALYSIS OF THE OXIDIZING AND REDUCING SIDES OF THE $O_2$-EVOLVING SYSTEM OF PHOTOSYNTHESIS

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## SUMMARY

Oxygen gushes in isolated spinach chloroplasts without added electron acceptor were observed *via* modulated rate and flash yield measurements. The results, analyzed with emphasis on the interactions of the oxidizing and reducing sides of System II, suggested the following:

1. The observed kinetics are consistent with the linear 4-step $O_2$ "clock" described by B. Kok, B. Forbush and M. McGloin (1970, *Photochem. Photobiol.* 11, 457–475), provided one accounts for the redox state of the System II acceptor Q.

2. The $O_2$ precursor (S) states function independently while the electron acceptor pools "Q–$A_2$" of different System II units interact. Thus, the oxygen flash yield is proportional to the product $[S_3]\cdot[Q_{ox}]$.

3. Primary acceptor Q reacts as one of a homogeneous pool ("$A_2$") of $\leq 5$ equivalents per trapping center which may represent two molecules of plastoquinone.

4. The secondary electron acceptor pool ("$A_1$"), observed after far-red preillumination or a long dark period, reacts with a high equilibrium constant with respect to Q. This pool also appears to contain about 5 equivalents per electron transport chain.

5. A cause of the apparent variability of pool sizes is discussed which is based on the lability of the $O_2$-evolving system and the interaction of the redox pools.

6. There appear to be two parallel leak paths (presumably to oxygen) located at the reducing sides of Systems I and II, respectively.

7. A very rapid deactivation of the $S_3$ state (approx. 0.5 s first half time) is observed under conditions in which the System II acceptor (Q) is reduced.

## INTRODUCTION

According to present concepts, the liberation of oxygen from water requires four sequential photoacts in a reaction center of Photosystem II ($S_0 \rightarrow S_4$ in the terminology of Kok *et al.*[1,2]). Consequently, the rate and flash yield of oxygen evolution are functions of the state of the electron donor sites. Only trapping centers in the $S_3$ state yield $O_2$ upon excitation.

In addition, the rate and flash yield of $O_2$ evolution are a function of the state of the electron acceptor sites of System II (Q in the terminology of Duysens and Sweers[3]). Only trapping centers in which Q is oxidized (and primary donor Z is reduced) can undergo a charge separation.

In isolated chloroplasts in the absence of an exogenous electron acceptor an $O_2$ "gush" is observed at the onset of illumination with short-wave light after a period of darkness or far-red light. This gush reflects the photoreduction by System II of an endogenous pool of reductant located between the two photoacts. After depletion of this pool the steady-state $O_2$ evolution rate is low and restricted by a slow reoxidation of presumably the primary electron acceptor of System I[4]. A number of studies have been made of this oxygen gush and of the reduction and oxidation of the endogenous electron acceptor pools. These were based on measurements of the rate of $O_2$ exchange[5,6], of the fluorescence yield[7–9], absorption changes of plastoquinone[10–12], and absorption changes of P700[13–15]. To date, these studies have not yielded a consistent picture; the size of the pool appears larger when viewed from the System II side (in $O_2$ or fluorescence measurements) than when observed *via* System I ($\Delta A$ at 700 nm or viologen reduction rate). Kinetically the pool, as observed *via* $O_2$ or fluorescence, is clearly inhomogeneous. Of the two fractions ($A_1$ and $A_2$)[6], Fraction "$A_2$" is more rapidly reoxidized in dark and shows a lower equilibrium constant in its reaction with primary photoreductant Q[9].

In this paper, we present an analysis of the interaction of the oxidizing and reducing sides of System II (*i.e.* the S states and redox pools) as viewed by $O_2$ evolution. A new aspect in our approach is the use of sequences of saturating 5-$\mu$s light flashes to analyze the state of the system during the gush. In this type of illumination events proceed in single equivalent steps, since each flash hits all traps in the system once. Each flash yield of $O_2$ reflects the number of traps in the state $Q_{ox}$–$S_3$ prior to the flash, which facilitates interpretation and analysis by computer modelling.

(In weak continuous light the momentary rate also reflects the number of $Q_{ox}$–$S_3$ states. However, calibration of the light flux in terms of hits per trap and per unit time is indirect and analysis is cumbersome.)

Techniques of measurement and procedures of chloroplast preparation are described in refs 1 and 16. Measurements were made at room temperature (20–25 °C) in a medium of 0.1 M KCl–0.05 M phosphate buffer (pH 7.5) in the absence of added electron acceptor. Chloroplast samples were diluted to 0.2 mg chlorophyll/ml from a stock suspension in 0.4 M sucrose–0.05 M Tris (pH 7.65). Uncoupling by methylamine had no detectable effect on the kinetics of $O_2$ evolution at the relatively low integrated light intensities used in these experiments.

## RESULTS AND INTERPRETATION

### *Kinetic analysis of the gush*

Fig. 1 presents the results of experiments in which saturating flashes (see ref. 1) were used to determine the momentary states of Photosystem II, and concomitantly to reduce the endogenous electron acceptor pools of System II. The two curves reflect essentially the same phenomenon since both 10 min dark and

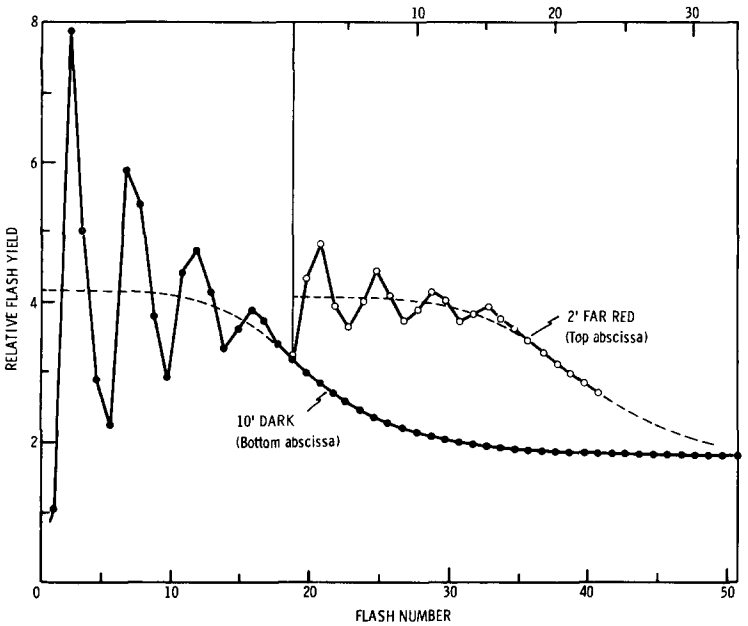


Fig. 1. Flash yield patterns (5 flashes/s) after (A) 10 min dark and (B) 2 min weak far-red illumination. The dashed line shows the computed "average" flash yield which, in the initial phase, corresponds to the steady-state flash yield which would be observed in the presence of an acceptor. The intensity of the far-red illumination ($\lambda \geq 700$ nm) was adjusted to achieve optimal pool restoration (*i.e.* high enough to sufficiently sensitize System I yet low enough so that the rate of System II sensitization was small compared to the Mehler rate).

2 min of far-red preillumination result in the oxidation of the pools. However, the far-red beam contained sufficient System II quanta to keep the $O_2$ system partially activated. Hence, compared to the curve observed after 10 min dark, the oscillations were much smaller, although sustained for an equal number of cycles.

If continuous modulated 650 nm light is used to convert the pools (see Fig. 2, top curve) essentially the same phenomena are observed except that the activation of the $O_2$ evolution system is reflected as an initial lag of the rate (rather than an oscillation of the yield).

These data show three distinct features:

(a) In continuous light given after long dark, the quantum yield is constant and maximal during the initial phase; *i.e.* the gush has a distinct cap. (The initial rise reflects the activation of the $O_2$ system.) In flashing light, this cap is reflected by an oscillation of the $O_2$ yield.

(b) At the end of this cap a "first-order" decrease in yield (or rate) occurs from the maximum down to a lower value. In flashing light, this decrease is accompanied by rapid damping of the flash yield oscillation.

(c) At the end of the first-order decrease a steady state is attained.

(1) *The high K pool* ($A_1$)

The initial constancy of the rate (or a "normal" oscillation of the $O_2$ yield during 1 or 2 cycles of a period of 4) suggests that chloroplasts contain an electron

acceptor pool which acts as an electron sink so that the primary electron acceptor Q remains fully oxidized, *i.e.* the electron acceptor(s) involved have a high equilibrium constant with respect to Q. (If Q were partially reduced the oscillation would be severely damped, as if an undue percentage of "misses"[1] occurred.)

Earlier observations[5,8,9] showed that this pool is slowly (approx. 5–10 min) oxidized in dark and more rapidly in far-red light; an estimate of its (apparent) equilibrium constant with respect to Q led to a value $K \simeq 10$ (ref. 9). In our present work we have noticed a day-to-day variation in this value ranging from approx. 10 (suggested by a slight down slope of the rate in the "cap") of the gush to "infinite" (suggested by perfect constancy of the rate during the cap).

The number of equivalents available in the $A_1$ pool is roughly indicated by the number of flashes (hits per trap) in the flat part of the gush. However, due to the flash yield oscillations, the end of the cap is somewhat hard to discern. In addition, a precise estimate of this number is complicated by several interferences which we will discuss in the following sections.

(2) *The low K pool* ($A_2$)

At the end of the "cap" of the gush (Fig. 1) the average $O_2$ yield decreases from the maximum to a low value $v_{ss}$. This decrease requires a number of flashes for completion and is roughly first order, suggesting the presence of an electron acceptor pool of several equivalents which reacts with a low equilibrium constant ($K_{eq}$ approx. 1) with the primary electron acceptor Q.

Fig. 2 illustrates $O_2$ gushes observed after dark periods of varying duration following preillumination. The height and duration of the gush increase with increasing dark time, implying that the redox intermediates involved become

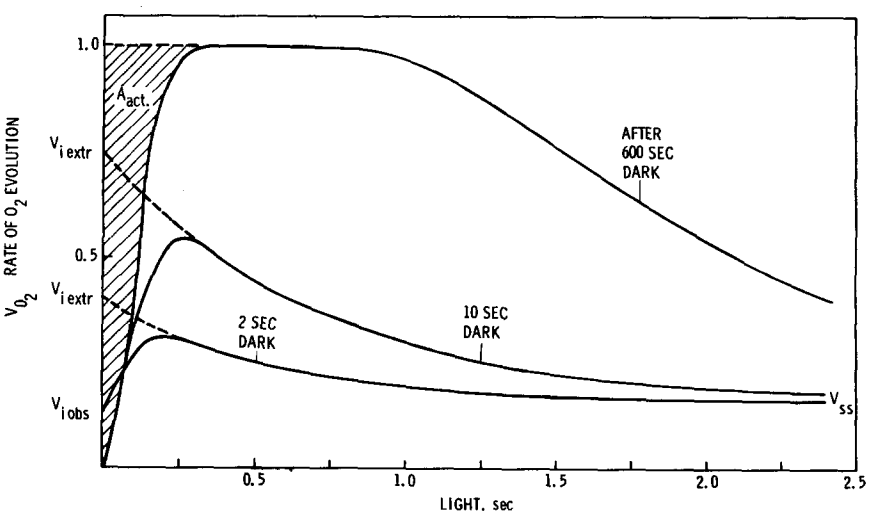


Fig. 2. ——, time courses of the modulated rate of $O_2$ evolution observed with modulated 650 nm light following dark periods of different duration; – – –, extrapolation of the rate to zero time assuming absence of deactivation and (for 2 s and 10 s dark) first-order time courses. Cross hatched area: "activation area" roughly corresponding to 1 photoconversion per trapping center[1].

progressively oxidized. (At the same time, the initial rate declines and becomes zero after approx. 10 s dark, so that a lag of the $O_2$ rate is observed.)

The same experiment can be performed with flashing light (Fig. 3). Again, with increasing dark time one observes an increase in the number of acceptor equivalents and a loss of $O_2$ precursor states. Note that as long as Q and $A_2$ are partially reduced no oscillation of the flash yield is observed; instead the yield rises and falls monotonically. Apparently, due to the partially reduced state of Q each flash converts only a fraction of the traps and thus the normal oscillation of the yield is suppressed (this is kinetically equivalent to a high value of $\alpha$, the fraction of misses).

Another experiment which demonstrates the effect of the state of the A pools on the oscillatory behavior of the $O_2$ system is shown by the open squares in Fig. 3. This experiment was performed with whole cells of *Scenedesmus* mutant No. 8 (ref. 17) which has no detectable System I activity. The deactivation of the $O_2$ system and the dark restoration of the $A_2$ pool in the mutant cells closely resembles that in chloroplasts. However, in contrast to chloroplasts the number of oxidized

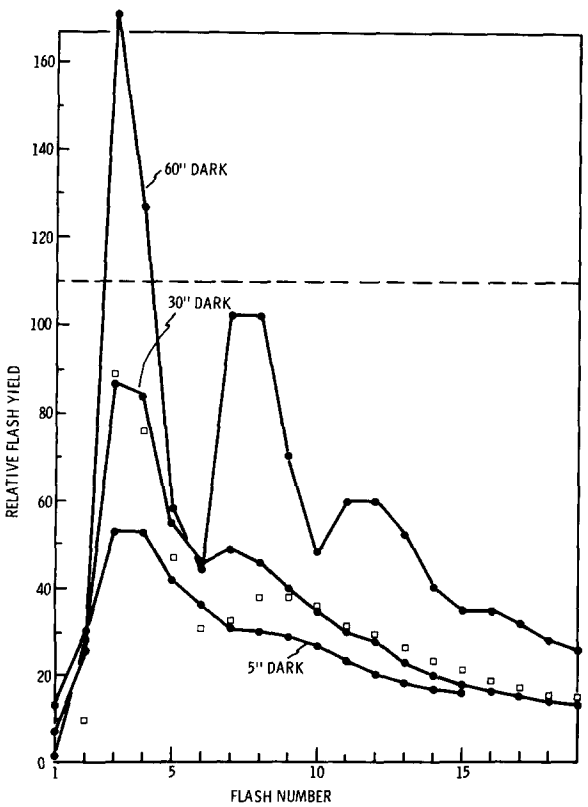


Fig. 3. Flash yield patterns (10 flashes/s) observed after 5 s, 30 s, and 60 s dark (●—●). Before each dark period the sample was subjected to a series of flashes to attain steady state. □, flash yield pattern observed after 10 min dark with whole cells of *Scenedesmus* mutant No. 8. See text for details. – – represents the "average" flash yield in the initial phase obtained in a parallel 10-min dark experiment (see Fig. 1).

pool equivalents reached a maximum after 30–60 s dark. This is illustrated in Fig. 3; the $O_2$ flash yield pattern after a long dark period (10 min) is very similar to that observed with chloroplasts after 30 s dark period. The $O_2$ system was completely deactivated but only the $A_2$ pool was restored and no $A_1$ pool was observed.

To separate these two dark processes (pool restoration and deactivation, *i.e.* the loss of $S_2$ and $S_3$) we assumed that the photoreduction of the low K pool proceeds continuously and first order in the light. This would imply that, without interference from deactivation, the initial rate in Fig. 2 would have had the value indicated as $v_{i\ extr}$. In Fig. 4, this extrapolated initial rate is plotted as a function of the area bounded by the (extrapolated) $O_2$ gush corrected for the steady-state rate. This area presumably is a measure of the redox pool oxidized in the dark[8,16].

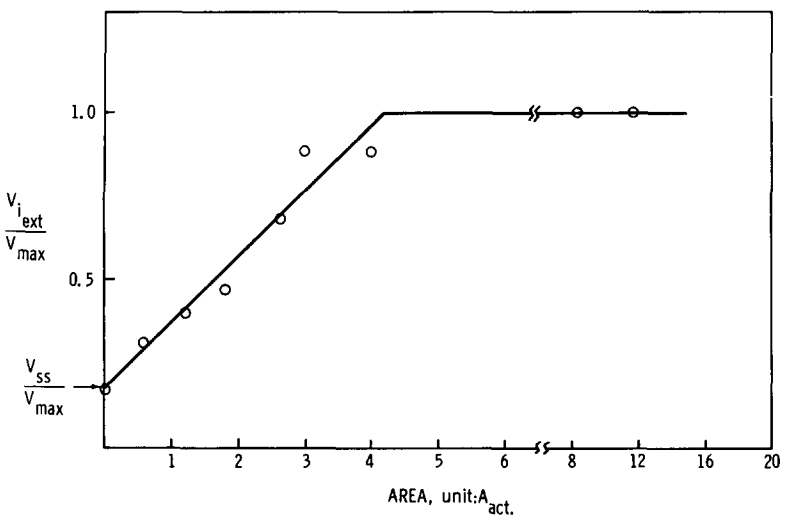


Fig. 4. Dependence of the extrapolated initial rate of $O_2$ evolution upon the area bounded by the gush. For this graph data similar to those shown in Fig. 2 were used.

We further assume that the complete activation of the $O_2$ system requires about 1 quantum per trapping center[1]. Then, the initial rise of the rate (the shaded area in Fig. 2) reflects the concentration of the trapping centers and can be used to calibrate the gush area in units of [Q]. Fig. 4, computed in this manner, shows a linear relation between $v_{i\ extr}$, the initial efficiency of System II, and a redox pool of about 5 equivalents per trapping center. The linear dependence of the extrapolated initial rate upon the area implies that the equilibrium constant between this pool and Q is close to unity (*e.g.* 1 Q and 4 $A_2$ is kinetically equivalent to 5 Q).

An alternate and more general computation of the magnitude of the pools is to analyze the $O_2$ rate (or yield) as a function of the number of equivalents added to the system. In the flash experiments each flash converts all $Q_{ox}$; *i.e.* it induces 1 transfer per trap if all Q is oxidized ($Q_{ox}=Q_{tot}$) and less if $Q_{ox}<Q_{tot}$ (*viz.* $(Q+A_2)_{ox}/(Q+A_2)_{tot}$). In continuous light, the activation rise can be used to compute how frequently the light beam hits each System II trap[2]. For instance, in the experiments shown in Fig. 2, the traps are hit once every approx. 0.2 s. For a first-order process the number of pool units $n$ (equivalents of Q) is given by $n=\exp(\ln 2/f)/[\exp$

$(\ln 2/f)-1]$, where $f$ is the number of flashes to attain half-maximal yield*. In the experiment shown in Fig. 1, after 2 min far red, the maximum flash yield is maintained for a duration of about 14 flashes after which the yield falls to one-half the maximum in another 10 flashes. On first sight this suggests that the $A_2$ pool in this experiment contains as much as 15 equivalents. However, as will be discussed later, we have reason to believe that this number is far too high.

(3) *The steady-state (Mehler) rate*

After the gush is completed, there remains a "leak rate" $v_{ss}$ which amounts to about 1 equivalent/s. One source of this leak might be inferred from the data presented in Fig. 2. From the increase of the extrapolated initial rate ($v_{i\ extr}$) in dark, it appears that roughly 4 pool equivalents were oxidized in 10 s (assuming a total pool of 5 equivalents). If this oxidation were entirely due to $O_2$ uptake[18] the rate constant $k_R$ for the autoxidation of this pool would be approx. 0.6 equivalent$\cdot s^{-1}$. Its first-order photoreduction locates this pool close to one of the primary photoreductants; the fact that it is not reduced by far-red light locates it close to Q.

The existence of a leak path *via* the System II reductants $Q-A_2$ can also be inferred from the fact that *Scenedesmus* mutant No. 8 exhibits a gush and a finite modulated $O_2$ rate and flash yield in the steady state (Fig. 3). Since X is presumably not functioning in this organism, any autoxidation must be on the System II side.

An alternate pathway for the restoration of the $A_2$ pool is the rapid deactivation described in the next section (a back reaction with $S_2$ and $S_3$). However, according to present concepts this path could account for only approx. 1 equivalent per trap and thus for only part of the restoration.

Data presented in Table I imply that in chloroplasts an oxidation of the reductant of System I (X in Scheme I) also contributes to the steady-state rate. In these experiments we used a 650-nm and a far-red modulated beam to run out the gush. The intensities were adjusted to equalize the activation lag and the rates of $O_2$ evolution during the cap of the gush ($v_{max}$). Therefore, System II was sensitized at the same rate in the two beams, but System I was activated much faster in the far-red beam. Table I shows that the rate of $O_2$ evolution in the steady state was significantly higher in the far-red beam than in the 650-nm light. This result is consistent with an interaction of X with $O_2$; since X is relatively more reduced in far-red light, its interaction with $O_2$ will be greater and the overall throughput of the system increased.

(4) *Rapid deactivation*

Two of the four intermediate oxidation states of the $O_2$-evolving enzyme ($S_2$ and $S_3$) are unstable and revert to $S_1$ as a result of the reducing action of uniden-

* Let $f$ = the number of flashes given to a system and $n$ = the number of acceptor molecules ($Q_{tot}$). Then the first flash will have a quantum yield of unity, will remove one equivalent of Q, and $n-1$ equivalents will remain. Since in general, the quantum yield is $Q_{ox}/Q_{tot}$ we find:

| *Flash number* | *equivalents removed* | *equivalents remaining* |
|---|---|---|
| 1 | 1 | $n-1$ |
| 2 | $(n-1)/n$ | $(n-1)-(n-1)/n=(n-1)^2/n$ |
| 3 | $(n-1)^2/n^2$ | $(n-1)^2/n-(n-1)^2/n^2=(n-1)^3/n^2$ |
| | *etc.* | |

Therefore, $n/2=(n-1)^f/n^{f-1}$; $\ln 2=f\ln[n/(n-1)]$ and $n=\exp(\ln 2/f)/[\exp(\ln 2/f)-1]$.

TABLE I

EFFECT OF COLOR ON $v_{ss}$

$v_{max}$ and $v_{ss}$ are the observed maximum and steady-state (Mehler) rates, respectively. The last column gives the number of 12-ms light pulses required to reach 63% ($1-e^{-1}$) of $v_{max}$ in the activation of $O_2$ evolution after 10 s dark. Light filters: 650 nm, interference filter (5 nm half width); far red, Schott RG-8 (10% transmittance at 688 nm); wire screens to equalize System II activity. The modulated light was administered in pulses of 12 ms spaced approx. 125 ms apart which improved the signal-to-noise ratio. The integrated light intensity was approximately 0.5 hit/s per System II trap.

| *No.* | *Light* | $v_{max}$ | $v_{ss}$ | *Number of pulses For $O_2$ activation to 63%* |
|---|---|---|---|---|
| 1 | 650 nm | 95 | 42 | 15 |
| 2 | Far red | 104 | 63 | 16 |
| 3 | 650 nm | 80 | 35 | 14 |

tified chloroplasts intermediates. It has been reported[1,2,19–21] that after preillumination the half life of the $S_3$ rate is a few seconds in whole algae and longer (>10 s) in isolated chloroplasts with added electron acceptor.

After 10 min of darkness the $O_2$-evolving system becomes completely deactivated, *i.e.* the observed initial $O_2$ rate is zero. If the light beam is interrupted for shorter periods of time (*e.g.* 2 s in Fig. 2), the $S_3$ state is incompletely deactivated as evidenced by the finite initial $O_2$ rate.

In Fig. 5 we plotted the ratio of the observed rate ($v_{i\ obsd}$) over the rate predicted in the absence of deactivation ($v_{i\ extr}$, see Fig. 2), which should reflect the amount of $S_3$ remaining after a given dark time. The time course of this rapid deactivation appears biphasic; the first half time is only approx. 0.6 s, some 20 times shorter than that observed in the presence of added electron acceptor. Although

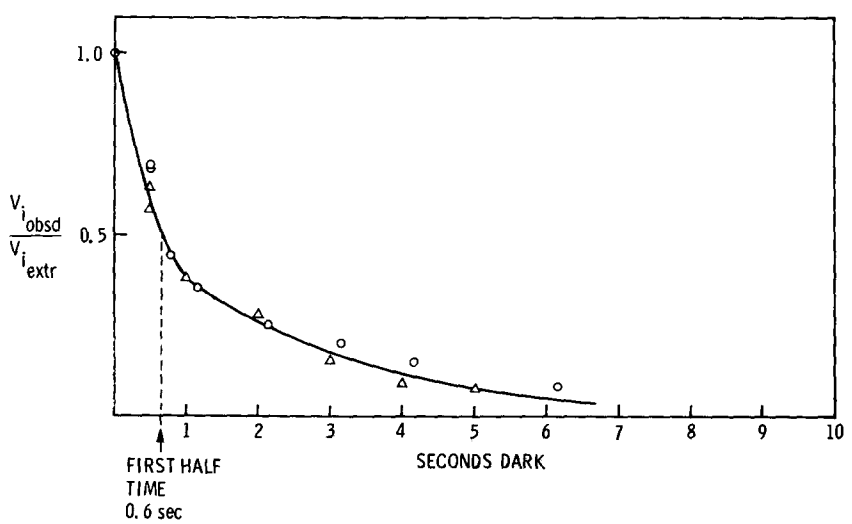


Fig. 5. The ratio $v_{i\ obsd}/v_{i\ extr}$ as a function of the dark time (after $v_{ss}$ was attained in preexposure, see Fig. 2). △ and ○ represent the results of two different experiments.

we cannot at this moment conceive of a consistent hypothesis, we correlate this difference with the reduced state of the A pools. In their oxidized form, these pools accept electrons from Q and thus may prevent backreaction with the $O_2$ precursor $S_3$.

The rapid decay in Fig. 5 closely resembles the decline of the fluorescence yield in darkness observed either under the conditions used in these experiments or in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU). For the latter case, Bennoun[22] suggested that a backreaction occurred between the photoproducts of System II.

If the observed rapid deactivation would only involve the $S_3$ state, the distribution of S states during the steady state (Mehler rate) would not be equal (0.25, 0,25, 0.25, 0.25). In this case, the skady-state flash yield $Y_{ss}$ would no longer precisely reflect the state of the A pools, $[A_{ox}]$ being higher than predicted by the $Y_{max}/Y_{ss}$ ratio. Since our observations do not reveal the precise nature of this rapid deactivation, we have not attempted a quantitative analysis. The uncertainty this introduces in our computations, however, is small.

*Analysis of the gush by computer modelling; measurements using an alternating flash-rate regimen*

By now, it might be clear that with several interacting processes involved, "eyeball" interpretations will be of limited use. We might briefly enumerate the various aspects to be reckoned with:

(1) After a dark period System I is operative (P700 reduced, X oxidized). Consequently, one equivalent can pass all the way through the chain.

(2) During and after the gush there are two leak paths, presumably to oxygen. This tends to increase the apparent size of the pools in a wavelength-dependent manner.

(3) The flash does not convert all System II traps; *i.e.* there is a certain percentage of "misses"[1,2] even under optimal conditions. In fact with aging of the sample, the System II efficiency for $O_2$ evolution tends to drop and the ratio of electron throughput between the two photosystems changes.

(4) Flash yield and rate are non-linearly related, the relation being dependent upon the state of Q and $A_2$[23]. Consequently, the rate shown in the tail of the gushes may not be an accurate reflection of the state of Q.

(5) The rapid deactivation observed with partially depleted pools alters the relationship of $O_2$ evolution to $Q_{ox}$.

To precisely evaluate the momentary relation between rate and yield we performed experiments in which the two illuminations were interlaced and their photochemical production recorded simultaneously. The dots connected by a solid line in Fig. 6 show the $O_2$ flash yields observed in a sequence of light flashes with a spacing of 0.2 s given after 10 min darkness. A weak monochromatic 650-nm beam was given for 12 ms immediately prior to each flash. Since very few photons were added during the 12-ms weak light pulses (just enough to measure the rate beyond the noise level), the photoevents were driven nearly exclusively by the flashes. The effect of the weak light pulses is noticeable mainly as a decrease in the amplitude of the $O_2$ yield oscillation (since the S states tend to become scrambled as if there were a large number of double hits).

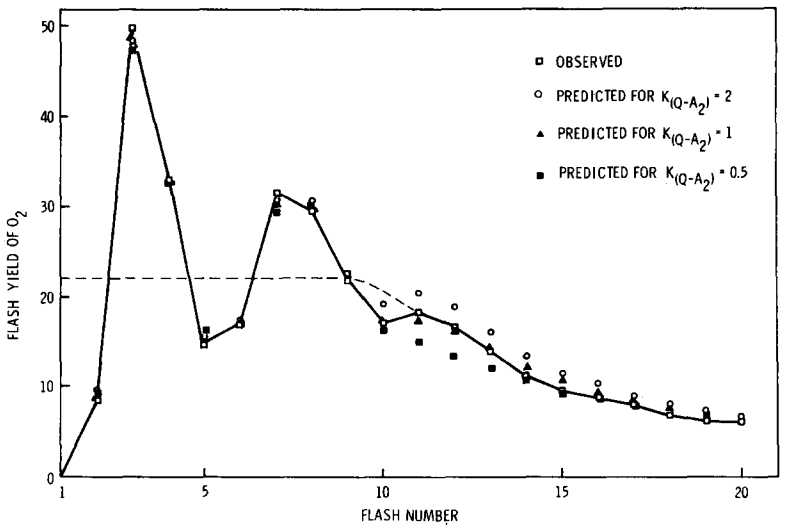


Fig. 6. Comparison of observed and computed $O_2$ flash yields (5 flashes/s), in a sequence of dark-rate-flash alternations given after 10 min of darkness. For the computation the following parametric values were used (see scheme, Fig. 8): $\alpha=0.15$; $\beta=0.05$ (ref. 1); $k_1=k_{-1}=k_2=k_3=100\ s^{-1}$ (ref. 13); $k_{-2}=0.1$; $k_{-3}=0$; $k_M=1\ s^{-1}$; $k_R=0$; $Q=1$; $A_2=4$; $A_2+Q=Q_{tot}$; $A_1=5$; $h\nu_{II}=5\ s^{-1}$, $h\nu_I=3.5\ s^{-1}$ (calculated from activation rise as described in text); at $t_0$, $S_0$: $S_1$: $S_2$:$S_3=25:75:0:0$. The computed values shown were derived assuming a single Mehler rate (*i.e.* $k_R=0$) and have a standard error $s=2.3$ (see text and Fig. 7). A similar computation assuming a dual Mehler rate number was also consistent with the observed flash yields ($s=2.0$). For this computation: $k_R=0.14$ (from Fig. 2), $k_M=0.4$, and $A_1=6$; the other parameters were the same as above .– –, see Fig. 1.

Fig. 7 shows a plot of the flash yield *vs* the $O_2$ rate observed prior to each flash. Note that for the first 10 flashes the yield and its preceding rate are linearly related. However, as soon as Q becomes partly reduced, the oscillations are quickly damped and the yield *vs* rate plot becomes decidedly non-linear. Experiments of this type yield the maximum and most coherent information and therefore have been used for computer modelling.

Fig. 8 clarifies our notation and illustrates the reaction scheme we have used to model the experiment shown in Figs 6 and 7. The solid triangles in Fig. 6 fit the observed values (open squares) quite well. Note that in a certain region of the curve, the fit is quite sensitive to the choice of $K_{(Q-A_2)}$. In contrast to the best fit value $K_{(Q-A_2)}=1$, equilibrium constants of 0.5 and 2 introduce deviations beyond experimental error.

Fig. 9 shows the effect of the variation of some of the computational parameters on the goodness-of-fit of a predicted *vs* observed flash-rate regimen. In this figure, the standard error $s$ is presented as a function of the variation about the nominal value of each parameter (see Fig. 6)*. The slope of each curve provides

* Although this analysis is a good overall indicator of the accuracy of the model, it is not foolproof, and sometimes the "educated eyeball" method is more instructive. Since different parts of the curve are sensitive to different parameters, in certain cases most of the deviation can be concentrated in one area of the curve (*cf.* Fig. 6). However, the standard error $s$ essentially averages this deviation over the whole curve, thus diluting its impact.

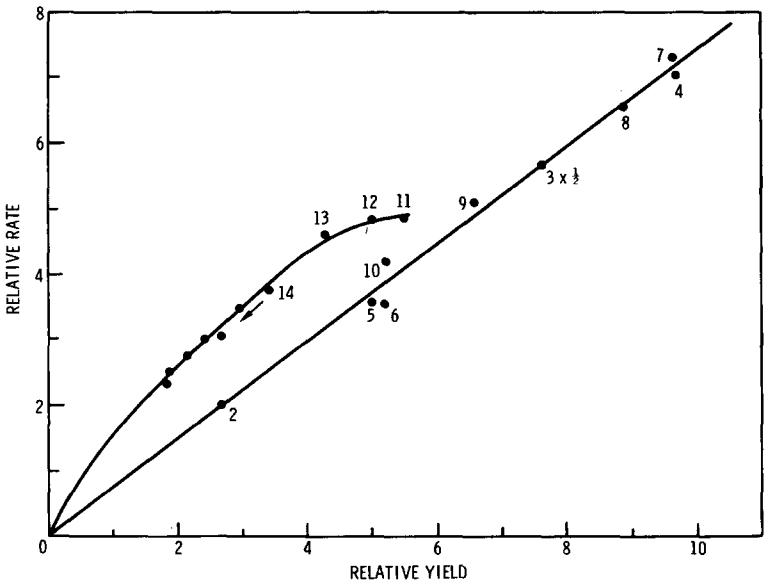


Fig. 7. Relative rate *vs* relative yield during the course of a gush by flashes. Data are from the experiment shown in Fig. 6. The numbers adjacent to the experimental points indicate the flash number in the sequence.

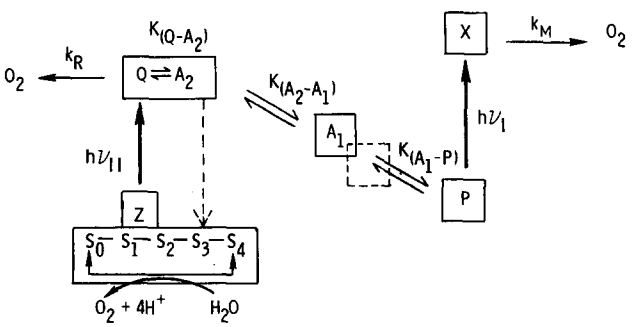


Fig. 8. Schematic representation of the reaction sequences used to model the experiment shown in Fig. 6. In this reaction scheme $h\nu_{II}$ and $h\nu_I$ are the rate constants (hits·trap$^{-1}$·s$^{-1}$) for the light reactions of Systems II and I, respectively. $K_{(Q-A_2)}$, $K_{(A_2-A_1)}$ and $K_{(A_1-P)}$ are the equilibrium constants for the Q–$A_2$, $A_2$–$A_1$ and $A_1$–P reactions, respectively; $K_{(Q-A_2)}=k_1/k_{-1}$; $K_{(A_2-A_1)}=k_2/k_{-2}$, and $K_{(A_1-P)}=k_3/k_{-3}$. $k_M$ and $k_R$ are the leak rates to oxygen from Systems I and II. Values for the constants are given in legend of Fig. 6.

an indication of the sensitivity of the computed fit to variation of a given parameter. (Although all curves are presented as continuous functions, the calculations for the $A_1$ and $A_2$ pool sizes were based only on integral values.)

Note that at point $N=1$ (the nominal "best fit"), $s=2.3$. By way of comparison, $s=2.3$ (coincidentally!) for two experiments performed on the same day (*i.e.* the results of the first experiment were used to "predict the results of the second"). Thus, the uncertainty in the fit of the model to the experiment is, in this instance, about equivalent to the experimental error.

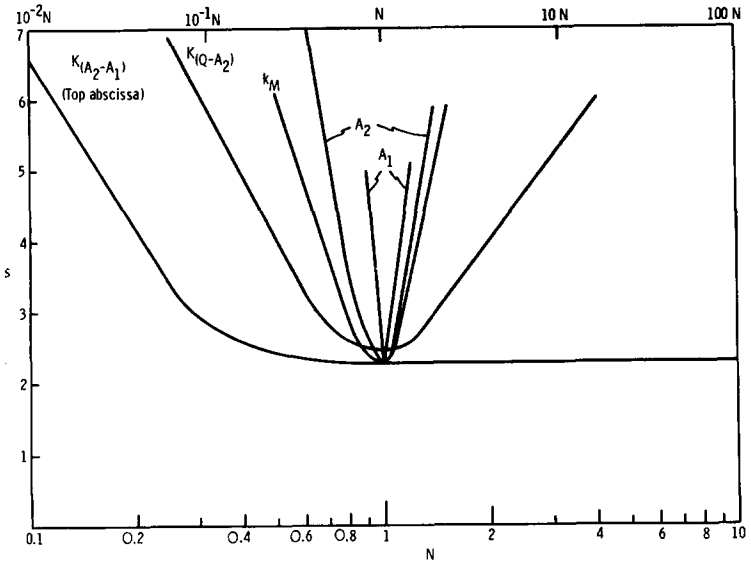


Fig. 9. Effect of variation of computational parameters on the goodness-of-fit. $s$ is the standard error of the computed *vs* observed values; $N$ is the derived best-fit value for each parameter. (1) Vary $K_{(Q-A_2)}$ with: Q=1, $A_2$=4, $K_{(A_2-A_1)}$=1000, $\alpha$=0.85, $k_1 \approx 100$, $k_{-1} \approx 100$, $k_M$=1, $N$=1.0. (2) Vary $K_{(A_2-A_1)}$ with: $A_2(\equiv Q)$=5, $A_1$=5, $k_M$=1, $N$=1000. (3) Vary $k_M$: $A_2$ ($\equiv$Q)=5, $A_1$=5, $K_{(A_2-A_1)}$=1000, $N$=1.0. (4) Vary $A_2$ ($\equiv$Q) with: $A_1$=5, $K_{(A_2-A_1)}$=1000, $k_M$=1.0, $N$=5. (5) Vary $A_1$ with: $A_2(\equiv Q)$=5, $K_{(A_2-A_1)}$=1000, $k_M$=1.0, $N$=5. Since $s$ is a weak function of the rate of Q–$A_2$ equilibration in the neighborhood of $k_1$ and $k_{-1}$=100, $s$=2.5 at $N$=1 for these values of the rate constants; as $k_1$ and $k_{-1}$ increase, $s$ approaches 2.3 at $N$=1.

To gain an intuitive feel for the significance of these curves we can compare the value of curve $K_{(Q-A_2)}$ at 0.5 $N$ and 2.0 $N$ with the computed values for $K_{(Q-A_2)}$ = 0.5 and $K_{(Q-A_2)}$=2 shown in Fig. 6. If we follow this criterion of a "good fit" we then conclude that (a) $K_{eq}$ for the reaction $Q \rightleftharpoons A_2$ is between 0.5 and 2.0; (b) the $A_1$ pool contains 5 equivalents; (c) the $A_2$ pools contain 4 to 6 equivalents, and probably 5; (d) $k_M$ is in the range 0.7 to 1.2 $s^{-1}$; (e) $K_{eq}$ for the reaction $A_2 \rightleftharpoons A_1$ is greater than 100.

Values for the parameters relating to the operating of the $O_2$ "clock" ($\alpha$, $\beta$, and the starting values of the S states) were taken from ref. 1 except for $\alpha$, which was determined empirically. Our calculations suggest that a model based on 2 Q's of widely different redox potential[24] is not consistent with observed phenomena. We have also tested and rejected the proposal[25] that X=5; there appears to be only one equivalent of X per electron transport chain.

## DISCUSSION

Several aspects of our analyses are worthy of note:

(1) The linear model for charge cooperation in $O_2$ evolution[1] fits the data within experimental error after incorporation of the redox state of Q. As Q becomes reduced the percent of misses ($\alpha$) increases and the flash yield decreases proportionately.

(2) Q interacts with the $A_2$ pool as if the equilibrium constant were about 1; this is kinetically equivalent to a homogeneous pool of 5 Q's.

(3) The significance of the apparent equilibrium constant between the $A_1$ pool and Q and its variability are not obvious. We suspect that even in weak light no true equilibrium is maintained.

(4) The kinetic analysis is quite sensitive to the overall Mehler rate, but does not distinguish well between a single or dual $O_2$ uptake path. If, in the experiment shown in Fig. 6, a "dual" Mehler had been assumed rather than a single one, the computed $A_1$ pool would have contained 6 rather than 5 equivalents.

(5) The incorporation of rapid deactivation "improves" the fit of the computed values, but not significantly compared to the experimental error. Consequently, we have not pursued this aspect of the analysis.

(6) Using the same parameters as in Fig. 6 and the transfer parameter $a=0.5$ of Joliot and Joliot[23], the model also successfully predicts the rates observed prior to the flashes ($s=2.2$). Some earlier determinations of the $O_2$ rate as a function of $[Q_{ox}]$ may have been subject to artifacts because the flash was superimposed on a steady-state rate (Myers, J., personal communication). The present experiments thus corroborate the non-linearity between the flash yield and the rate of $O_2$ evolution.

(7) A comparison of the data shown in Figs 1 and 6 show that, although the apparent pool sizes and Mehler rates differ significantly, the yields of the first 4–5 flashes oscillate quite similarly. These results are consistent with the concept that (a) the $O_2$-evolving centers (the S states) function independently (see 1) and (b) electrons can be transferred from one System II unit to another (*i.e.* the $Q$–$A_2$ pools interact). Cross transfer of electrons between A pools of different photosynthetic chains has been shown by the observations of Siggel *et al.*[26]. Thus, active System II units can deplete the redox pools of adjacent "dead" units. In our (relative) measurements* this leads to a longer "cap" (or a longer sustained oscillation), a slower decline of the gush and a larger Mehler rate (compared to $v_{max}$) in preparations with low System II specific activity.

(8) In Expt 6 both the $A_1$ and $A_2$ pools appear to contain about 5 electron equivalents. As mentioned above we ascribe the variability of the apparent pool sizes as viewed *via* System II (*cf.* Fig. 1 and refs 5–12) to a loss of activity of the $O_2$-evolving system, probably the most labile component of the photosynthetic apparatus. For example, if one-half of the $O_2$-evolving units were inactive, the observed pools would appear to be twice too large. Accordingly, the smallest observed pool is the "best result". The present analysis thus suggests that the $A_1$ and $A_2$ pools each contain at most approx. 5 equivalents.

(9) Presently, it is difficult to identify the chemical nature of Q, $A_2$ and $A_1$. Our analysis suggests that Q and $A_2$ are chemically identical — possibly representing 2 molecules of plastoquinone. On the other hand, the $A_1$ pool appears to be distinctly different, is not intimately related to System II, and might not be homogeneous. Although not readily reconciled with earlier reports[12,27] Bishop and Wong[28] found that algae contained only plastoquinone A in a concentration of 1 molecule per

* The measurement of the System II specific activity is not feasible in these experiments since (1) all $O_2$ measurements made on the electrode are relative; and (2) significant System II activity can be lost on the electrode during the course of a 10–30-min experiment.

200 chlorophylls, which implies about 4 electron equivalents per System II trap. This would suggest that the $Q-A_2$ pool is plastoquinone while the $A_1$ pool consists of other redox compounds.

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