Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 1834–1838, March 1993
Biophysics

Oxygen evolution in photosynthesis: From unicycle to bicycle

(photosystem II/S states/quinone acceptors/misses)

VLADIMIR P. SHINKAREV* AND COLIN A. WRAIGHT†

Department of Plant Biology, University of Illinois, Urbana, IL 61801

Communicated by Jack Meyers, October 30, 1992 (received for review August 10, 1992)

ABSTRACT **Flash-induced oxygen evolution in the thylakoids of plants and algae exhibits damped oscillations with period four. These are well described by the S-state model of Kok *et al.* [Kok, B., Forbush, B. & McGloin, M. (1970) *Photochem. Photobiol.* 11, 457–475], with damping provided by empirical misses and double hits in the reaction center of photosystem II. Here we apply a mechanistic interpretation of misses as mainly determined by reaction centers that are inactive at the time of the flash due to the presence of either P^+ or Q_A^-, according to the electron transfer equilibria on the donor and acceptor sides of the reaction center. Calculation of misses on this basis, using known or estimated values of the equilibrium constants for electron transfer between the S states and tyrosine Y_Z, between Y_Z and P680, as well as between the acceptor plastoquinones, allows a natural description of the flash number dependence of oxygen evolution. The calculated misses are different for each flash-induced reaction center transition. Identification of this mechanism underlying the miss factor for each transition leads to the recognition of two different reaction sequence cycles of photosystem II, with different transition probabilities, producing an intrinsic heterogeneity of photosystem II activity.**

In photosystem II (PSII), light activates electron transfer from the primary donor (P680) to the primary quinone acceptor (Q_A) and then to the secondary quinone acceptor (Q_B). Q_A is a one-electron carrier, whereas the secondary quinone acceptor Q_B can accept two electrons. Plastoquinol (Q_BH_2), generated after two turnovers of PSII, can exchange with an oxidized molecule of the plastoquinone pool and the acceptor quinone complex returns to the initial state (Q_AQ_B) with both quinones in the oxidized state (e.g., ref. 1). The "two-electron gate" character of Q_B, the kinetic stability of the Q_B semiquinone, and the exchange of neutral forms of the quinone result in flash number-dependent binary oscillations of the semiquinones (e.g., ref. 2).

Activation of PSII by single turnover actinic flashes leads to oxygen evolution with periodicity of four (reviewed in ref. 3). This periodicity was originally explained by introducing the S states (S_n) of the oxygen-evolving complex, where each S state has a different number (n = 0, 1, 2, 3, 4) of oxidizing equivalents (4). The dominant dark-stable state was S_1, resulting in maximal oxygen yield after three flashes. Later, Joliot and Kok (3) considered four photoactive states including the PSII reaction center (RC), Y_ZP680Q, and the O_2-evolving enzyme as components. Since then, however, many authors have returned to the original interpretation, where only the accumulated charges on the donor side are considered (for reviews, see refs. 5–9).

Damping of the period four oscillations was explained empirically by misses and double hits (4), the nature of which has never been made fully explicit. The double hit parameter is mainly attributed to a double turnover of the RC induced during the tail of the actinic flash (3, 4).

The nature of misses has not been mechanistically defined (see, however, ref. 9). In the analysis of Joliot and Kok (3), misses were suggested to be due either to the fraction of RCs in which a photochemical transition does not occur or to back-reactions that annihilate the effect of the previous flash. Although equal misses for each transition give adequate fitting of the observed oxygen evolution, many authors have suggested that they may be different for each S state (e.g., refs. 3, 9–12) and, indeed, some improvement of the fit is seen. However, these models have been essentially phenomenological in nature.

Here we suggest that misses are substantially determined by the fraction of RCs that have either P^+ or Q_A^- before each flash, due to the reversibility of the electron transfer reactions. With this underlying mechanism, the miss factor becomes a fundamentally informative parameter for the oxygen-evolving process. Calculation of misses from this standpoint, using available values for the equilibrium constants, gives good predictions for flash-induced oxygen evolution. The most important outcome, however, is recognition of two *different* reaction cycles with different transition probabilities and, consequently, different oxygen yield patterns in a flash series. The relative contributions of the two cycles depend on the initial conditions. This gives rise to an intrinsic, kinetic heterogeneity of PSII activity, which may contribute to the large number of heterogeneities derived from phenomenological analysis (e.g., ref. 13), only a few of which have been shown to have a structural foundation (e.g., refs. 14 and 15).

RESULTS AND DISCUSSION

From Kok Unicycle to Bicycle. In principle, the behavior of PSII RCs can be described by considering the states generated by an ordered sequence of the electron-transferring components on the donor and acceptor sides:

$$S\ Y_Z\ P\ I\ Q_A\ Q_B, \qquad [1]$$

where, in addition to Q_A and Q_B, S is the oxygen-evolving complex, which can accumulate four oxidizing equivalents; Y_Z is the tyrosine [TyrD1-161 (16, 17)] electron donor to $P680^+$; P is the primary electron donor, P680; and I is the electron acceptor pheophytin.

After each flash, the photogenerated hole is transferred from $P680^+$ to Y_Z and then to the Mn-containing oxygen-evolving complex (reviewed in refs. 5–9). Because the flash-induced transitions occur in the nanosecond–microsecond time range, we can assume that after each flash the relevant

---

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PSII, photosystem II; RC, reaction center.

*On leave from Biophysics Section, Department of Biology, Moscow State University, Moscow, Russia.

†To whom reprint requests should be addressed at: Department of Plant Biology, University of Illinois at Urbana-Champaign, 190 PABL, 1201 West Gregory Drive, Urbana, IL 61801-3838.

reactions reach quasi-equilibrium on the time scale of milliseconds and seconds. The flash-induced transitions on the donor side RC can then be represented as

$$\begin{tikzcd}
\boxed{S_0YP^+ \overset{K^0_{YP}}{\rightleftharpoons} S_0Y^+P \overset{K_{0Y}}{\rightleftharpoons} S_1YP} \arrow[r, "h\nu"] & \boxed{S_1YP^+ \overset{K^1_{YP}}{\rightleftharpoons} S_1Y^+P \overset{K_{1Y}}{\rightleftharpoons} S_2YP} \arrow[d, "h\nu"] \\
\boxed{S_3YP^+ \underset{K^3_{YP}}{\rightleftharpoons} S_3Y^+P \underset{K_{3Y}}{\rightleftharpoons} S_4YP \overset{\nearrow O_2}{\underset{K_{40}}{\rightleftharpoons}} S_0YP} \arrow[u, "h\nu"] & \boxed{S_2YP^+ \underset{K^2_{YP}}{\rightleftharpoons} S_2Y^+P \underset{K_{2Y}}{\rightleftharpoons} S_3YP} \arrow[l, "h\nu"']
\end{tikzcd} \qquad [2]$$

Here K^n_{YP} is the equilibrium constant of the transition: $S_nYP^+ \rightleftharpoons S_nY^+P$ ($n = 0, 1, 2, 3$); K_{nY} is the equilibrium constant of transition: $S_nY^+P \rightleftharpoons S_{n+1}YP$; and K_{40} is equilibrium constant for the reaction: $S_4YP \rightleftharpoons S_0YP$.

For the time being, we do not consider the slow transitions of Y_D [TyrD2–160 (18, 19)], an alternate donor, in the second-minute time domain, which can reduce the oxygen-evolving complex in some S states (reviewed in refs. 5–8).

Each flash also activates the complex of quinone acceptors according to the following simplified scheme:

$$\boxed{Q_A^-Q_B \overset{M^1_{AB}}{\rightleftharpoons} Q_AQ_BH_2 \overset{M^2_{AB}}{\rightleftharpoons} Q_AQ_B} \underset{h\nu}{\overset{h\nu}{\rightleftharpoons}} \boxed{Q_A^-Q_B \overset{L_{AB}}{\rightleftharpoons} Q_AQ_B^-} \quad [3]$$

Pheophytin, I, may also be incorporated in this scheme but is omitted for clarity.

Light-induced electron transfer occurs from the donor side to the acceptor side of the RC and necessitates parallel consideration of the transitions on both sides. The fast equilibrating states in Schemes 2 and 3 can be designated by the letter D for the donor side and by the letter A for the acceptor side. If we consider D as having four stable oxidation states (D^0, D^+, D^{2+}, D^{3+}) and A with two states (A, A^-), then two different schemes arise by combining the transitions of donor and acceptor sides of the RC:

$$\begin{tikzcd}
D^+A \arrow[r, "h\nu"] & D^{2+}A^- \arrow[r, "h\nu"] & D^{3+}A \arrow[r, "h\nu"] \arrow[d] & DA^- \arrow[lll, bend left, "h\nu"] & \text{(Cycle V)} \quad [4] \\
& & O_2 & &
\end{tikzcd}$$

$$\begin{tikzcd}
D\,A \arrow[r, "h\nu"] & D^+A^- \arrow[r, "h\nu"] & D^{2+}A \arrow[r, "h\nu"] & D^{3+}A^- \arrow[lll, bend left, "h\nu"] \arrow[r] & O_2 & \text{(Cycle W)} \quad [5]
\end{tikzcd}$$

Each state is, in fact, a quasi-state obtained through equilibrium among the many elementary states of the RC, due to reactions on the donor (horizontal transitions) or acceptor (vertical transitions) sides of the RC, e.g.:

$$DA = \boxed{\begin{array}{ccccccc}
S_3YP^+Q_AQ_B & \rightleftharpoons & S_3Y^+PQ_AQ_B & \rightleftharpoons & S_4YPQ_AQ_B & \rightleftharpoons & S_0YPQ_AQ_B \\
\upharpoonleft\downharpoonright & & \upharpoonleft\downharpoonright & & \upharpoonleft\downharpoonright & & \upharpoonleft\downharpoonright \\
S_3YP^+Q_AQ_BH_2 & \rightleftharpoons & S_3Y^+PQ_AQ_BH_2 & \rightleftharpoons & S_4YPQ_AQ_BH_2 & \rightleftharpoons & S_0YPQ_AQ_BH_2 \\
\upharpoonleft\downharpoonright & & \upharpoonleft\downharpoonright & & \upharpoonleft\downharpoonright & & \upharpoonleft\downharpoonright \\
S_3YP^+Q_A^-Q_B^- & \rightleftharpoons & S_3Y^+PQ_A^-Q_B^- & \rightleftharpoons & S_4YPQ_A^-Q_B^- & \rightleftharpoons & S_0YPQ_A^-Q_B^-
\end{array}} \quad [6]$$

$$D^+A^- = \boxed{\begin{array}{ccccc}
S_0YP^+Q_A^-Q_B & \rightleftharpoons & S_0Y^+PQ_A^-Q_B & \rightleftharpoons & S_1YPQ_A^-Q_B \\
\upharpoonleft\downharpoonright & & \upharpoonleft\downharpoonright & & \upharpoonleft\downharpoonright \\
S_0YP^+Q_AQ_B^- & \rightleftharpoons & S_0Y^+PQ_AQ_B^- & \rightleftharpoons & S_1YPQ_AQ_B^-
\end{array}} \quad [7]$$

etc.

The quasi-states in cycles V and W are valid only after attainment of equilibrium among the elementary RC states. This occurs *in vivo* beginning from about 1 ms (1, 7, 9).

In the well-known Kok model for S-state transitions of the oxygen-evolving complex

$$\begin{tikzcd}
S_0 \arrow[r, "\gamma"] & S_1 \arrow[r, "\gamma"] & S_2 \arrow[r, "\gamma"] & S_3 \arrow[r, "\gamma"] & S_4 \arrow[llll, bend left, "\text{fast}"] \arrow[d] \\
& & & & O_2
\end{tikzcd} \qquad [8]$$

all transition probabilities, γ, have the same value. It has been common to define the S-state cycle in terms of misses (α), which can be defined as $(1 - \gamma)$ in the limit of zero double hits. If cycles V and W all have *equal* transition probabilities then they degenerate to a *single* Kok type cycle. However, the transition probabilities are necessarily *different* for cycle V and cycle W and they do not converge to a single kinetic cycle.

Dependence of Misses on Flash Number. Schemes **4** and **5** consider only the case when flashes induce sequential transitions of the RC. Kok's model also considers transitions between RC states differing by two oxidizing equivalents on the donor side of the RC (double hits). These are attributed to a double turnover of the RC, induced during the tail of the actinic flash, and are enhanced by oxidation of the iron atom (Q_{400}; see ref. 20) of the quinone acceptor complex. In principle, they can be minimized (21) or even eliminated (22), and for clarity we will not consider them here. However, they can easily be incorporated in cycles V and W. Focusing on misses, therefore, we first consider cycle W:

$$\begin{tikzcd}
\underset{(1)}{DA} \arrow[r, "\gamma_0^w"] & \underset{(2)}{D^+A^-} \arrow[r, "\gamma_1^w"] & \underset{(3)}{D^{2+}A} \arrow[r, "\gamma_2^w"] & \underset{(4)}{D^{3+}A^-} \arrow[lll, bend left, "\gamma_3^w"] \arrow[r] & O_2
\end{tikzcd} \qquad [9]$$

The transition probabilities γ_k^w ($k = 0, 1, 2, 3$) are proportional to the probability that the RC is in the state with reduced primary donor and oxidized primary plastoquinone acceptor:

$$\gamma_k \propto [\mathrm{P680}\ Q_A]_{\text{before flash}} \qquad [10]$$

This can be determined from the equilibrium within the quasi-states, such as **6** and **7**. Prior to equilibration, misses can arise from charge recombination, as suggested by Joliot and Kok (3) and Bouges-Bocquet (9). This source of misses can be dealt with by a kinetic extension of the quasi-equilibrium model presented here and will be elaborated elsewhere. Here, we wish to emphasize the contributions from the inherent equilibria on the donor and acceptor sides of the RC and the consequences of the parallel cycles, V and W.

In the quasi-state

$$D^+A^- = \boxed{\begin{array}{ccccc}
S_0YP^+Q_A^-Q_B & \overset{K^0_{YP}}{\rightleftharpoons} & S_0Y^+PQ_A^-Q_B & \overset{K_{0Y}}{\rightleftharpoons} & S_1YPQ_A^-Q_B \\
\upharpoonleft\downharpoonright L_{AB} & & \upharpoonleft\downharpoonright L_{AB} & & \upharpoonleft\downharpoonright L_{AB} \\
S_0YP^+Q_AQ_B^- & \underset{K^0_{YP}}{\rightleftharpoons} & S_0Y^+PQ_AQ_B^- & \underset{K_{0Y}}{\rightleftharpoons} & S_1YPQ_AQ_B^-
\end{array}} \quad [11]$$

a light-induced transition can occur only in states $S_0Y^+PQ_AQ_B^-$ and $S_1YPQ_AQ_B^-$, all other states having oxidized P680 or reduced Q_A. The relative concentration of these two states can be calculated considering equilibrium in the quasi-state of Eq. **11**:

$$\gamma_1^w = (1 - e^{-\phi I})\,PQ_A \simeq (1 - e^{-\phi I})\,P{\cdot}Q_A \overset{\phi I>>1}{\simeq} \frac{K^0_{YP}(1 + K_{0Y})}{1 + K^0_{YP}(1 + K_{0Y})}\cdot\frac{L_{AB}}{1 + L_{AB}}, \qquad [12]$$

where ϕ is the quantum yield of charge separation, I is intensity of the flash, and PQ_A is the relative concentration of the RC states with reduced P680 and oxidized Q_A, normalized to the concentration of all elementary states of the quasi-state [11]. So long as the electron transfer equilibria on the donor and acceptor sides occur independently, $[PQ_A] \simeq [P]\cdot[Q_A]$.

Expressions similar to Eq. **12** can be derived for the transition probabilities γ_i^w and γ_k^v between all RC quasi-states in cycles W and V. Values for some of the equilibrium constants necessary to calculate the transition probabilities are available or can be estimated from the literature. However, it must be admitted that discrepancies exist between different methods for determining the constants. The most marked of these comes from comparison of the rates of recombination of $S_2Q_A^-$ and $P^+Q_A^-$, which can be taken to indicate an overall equilibrium constant for $S_1Y_ZP^+ \leftrightarrow S_2Y_ZP$ of $\simeq 10^3$ (9). However, the decay kinetics of both states are widely reported to be polyphasic (e.g., ref. 23) and vary substantially in different preparations. Some representative values for the equilibrium constants of individual donor side reactions are listed in Table 1. The important point is that assays of P680$^+$ recovery show a measurable fraction of P680 to remain oxidized after a flash and that this fraction is larger after the second and third flashes (26, 28, 30). Thus, K_{YP}^2 and K_{YP}^3 are not large. Many of the equilibrium constants on the donor and acceptor sides of RCs also depend on pH, giving rise to pH dependence in the transition probabilities for cycles V and W. However, this does not simply reflect the measured proton-release stoichiometries of the water-oxidizing processes, which are associated with the net transitions between S states (e.g., ref. 31).

The difference between the two cycles is most evident from comparison of misses rather than transition probabilities. Fig. 1 shows the misses calculated in V and W cycles for transitions $D^0 \rightarrow D^{1+}$, $D^{1+} \rightarrow D^{2+}$, $D^{2+} \rightarrow D^{3+}$, and $D^{3+} \rightarrow D^0$, respectively. The intrinsic miss factors (i.e., $\phi I \rightarrow \infty$) differ manyfold for the two cycles.

Hence, there are two *different* period four cycles for RCs of PSII, functioning with different transition probabilities. This may be considered a type of phase-related heterogeneity of PSII RCs.

Time Dependence of the RC Transitions Between Flashes. As suggested by Joliot and Kok (3), misses may be due either to the fraction of RCs in which photochemical transitions do not occur or to reactions that annihilate the effect of light. We consider now the latter case. In the previous analysis, we assumed that there are no transitions between different quasi-states of the RC (defined as sets of fast-equilibrating elementary states) except those induced by light. We now generalize this to include transitions of the RC during the time between flashes, thereby incorporating slow relaxations of the S states of the oxygen-evolving complex and oxidation of the plastoquinone acceptor complex.

Table 1. Values of the equilibrium constants of different transitions on the donor and acceptor sides of RC

Transition		Equilibrium constant	Ref(s).
Donor side of RC			
$Y_ZP680^+ \leftrightarrow Y_Z^+P680$			
(inactive in O_2 evolution)	K_{YP}	18–600 (pH 5–7.5) 45 (pH 7)	24 25
(S_0)	K_{YP}^0	30 (pH 7.5)	26
(S_1)	K_{YP}^1	30 (pH 7.5) 7 (pH 7.5)	26 27
(S_2)	K_{YP}^2	2–5 (pH 7.5)	26, 28
(S_3)	K_{YP}^3	2–5 (pH 7.5)	26, 28
$S_0Y_Z^+ \leftrightarrow S_1Y_Z$	K_{0Y}	$>10^2$	5
$S_1Y_Z^+ \leftrightarrow S_2Y_Z$	K_{1Y}	9 (pH 7.5)	22
$S_2Y_Z^+ \leftrightarrow S_3Y_Z$	K_{2Y}	5 (pH 7.5)	22
$S_3Y_Z^+ \leftrightarrow S_4Y_Z$ $S_4Y_Z^+ \leftrightarrow S_0Y_Z$	$K_{3Y}\cdot K_{40}$	65 (pH 7.5)	22
Acceptor side of RC			
$Q_A^-Q_B \leftrightarrow Q_AQ_B^-$	L_{AB}	3.5–95 (pH 8.5–6)	29
$Q_A^-Q_B^- \leftrightarrow Q_AQ_BH_2$ $Q_AQ_BH_2 \leftrightarrow Q_AQ_B$	M_{AB}^1 M_{AB}^2	$>10^2$ (pH 7)	1

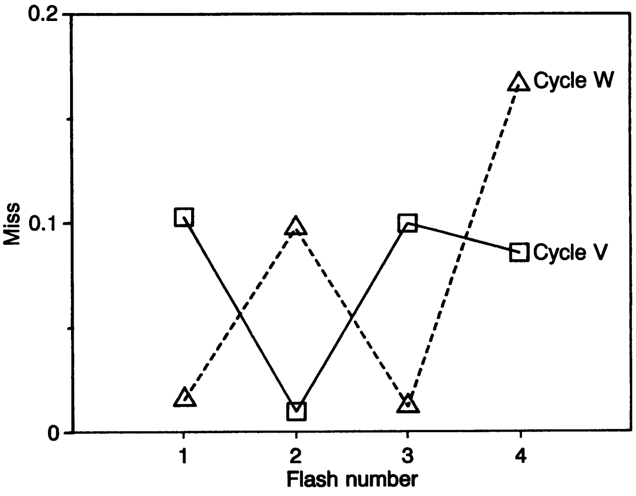

FIG. 1. Values of intrinsic misses (i.e., for 100% flash saturation) in cycles V and W for transitions $D^0 \rightarrow D^{1+}$, $D^{1+} \rightarrow D^{2+}$, $D^{2+} \rightarrow D^{3+}$, and $D^{3+} \rightarrow D^0$, respectively, calculated from Eq. **12** and similar expressions for γ_0, γ_2, and γ_3. The equilibrium constants were as listed in Table 1, with $L_{AB} = 9.4$, $K_{YP}^0 = 30$, $K_{YP}^1 = 30$, $K_{YP}^2 = 4$, and $K_{YP}^3 = 4$.

For return of the electron from the acceptor side to the donor side (recombination), the dark relaxation occurs within a given cycle. However, uncorrelated reactions—e.g., with exogenous oxidants and reductants—will induce transitions between quasi-states of different cycles and will mix cycles V and W (for clarity the states DA and DA$^-$ are shown twice):

$$\begin{array}{l}
\qquad\quad (1) \qquad\quad (2) \qquad\qquad (3) \qquad\qquad (4) \qquad\quad (1) \\
\qquad\quad DA \leftarrow D^+A^- \quad D^{2+}A \leftarrow D^{3+}A^- \quad DA \quad \text{(cycle W)} \\
\quad\nearrow \qquad \uparrow \swarrow \quad \uparrow \qquad \nearrow \ \uparrow \qquad \swarrow \ \uparrow \qquad \nearrow \\
DA^- \quad D^+A \leftarrow D^{2+}A^- \quad D^{3+}A \quad DA^- \quad \text{(cycle V)} \\
\ (8) \qquad\ \ (5) \qquad\quad (6) \qquad\qquad (7) \qquad (8)
\end{array} \qquad [13]$$

Here the vertical arrows indicate reduction of the donor side, the diagonal arrows indicate oxidation of the quinone acceptor complex, and the horizontal arrows indicate back-reactions. The extent to which the two cycles mix also depends upon endogenous (e.g., Q_{400}, the iron atom of the acceptor quinone complex, and Y_D, a second tyrosine donor to P680$^+$) as well as exogenous factors and on the flash repetition rate. In practice, involvement of Y_D can be minimized by use of flash repetition rates faster than about 1 s^{-1} (32). The time dependence of the mixing can be modeled explicitly, in the same manner as described for the period two oscillations of the bacterial acceptor quinone complex (e.g., ref. 33).

The existence of two entire and separate cycles in charge accumulation by PSII changes the description of all processes occurring in PSII. We now consider the consequences of our model for the behavior of the quinone acceptors, oxygen evolution, and delayed fluorescence. A significant fraction of RCs of PSII does not exhibit binary oscillations and does not evolve oxygen under normal experimental conditions (e.g., refs. 14 and 15). Obviously these are excluded from our analysis.

Binary Oscillations of Q_B^-. In spite of the similar two-electron nature of Q_B in PSII and bacterial RCs, the dependence of misses on flash number, with periodicity of four, immediately indicates a more complex behavior of Q_B^- in PSII. The quantum yield of Q_B^- generation is modulated by the dependence of P^+ on flash number. For example, for the values of equilibrium constants used above (Table 1), and for 95% flash saturation, the transition probabilities of the acceptor side will be

$$\begin{tikzcd}(Q_AQ_B) \arrow[r, "0.94"] & (Q_AQ_B)^- \arrow[r, "0.85"] & (Q_AQ_B) \arrow[r, "0.87"] & (Q_AQ_B)^- \arrow[lll, bend left, "0.84"]\end{tikzcd} \quad \text{(Cycle V)} \qquad [14]$$

$$\begin{tikzcd}(Q_AQ_B) \arrow[r, "0.94"] & (Q_AQ_B)^- \arrow[r, "0.85"] & (Q_AQ_B) \arrow[r, "0.94"] & (Q_AQ_B)^- \arrow[lll, bend left, "0.78"]\end{tikzcd} \quad \text{(Cycle W)} \qquad [15]$$

Hence, to fully describe the behavior of Q_B it is necessary to indicate the state of the oxygen-evolving complex.

Oxygen Evolution. Fitting oxygen flash yield patterns by the Kok model is frequently done with initial conditions $S_0 = 25\%$ and $S_1 = 75\%$, although this is partially due to the artifact of one-time donation from Y_D to the donor side charge accumulator (32, 34). If one assumes that after dark adaptation the quinone acceptors are oxidized, these initial conditions refer to the different cycles: 75% to the V cycle and 25% to the W cycle (see Schemes **4** and **5**). Thus, the experimentally observed oxygen evolution must be fitted with a mixture of the two independent cycles. However, under optimal conditions for oxygen evolution (pH 6.5–7.5), both cycles predict similar flash yield patterns and good fits can be obtained within each independent cycle, with the initial conditions of 25% D^0 and 75% D^{1+} (e.g., Fig. 4*A*). Fig. 2 shows the fit of cycle V to the experimental flash yield pattern, using the equilibrium constants given in Table 1, taken from the literature. For best fit to these experimental data, 10% double hits were included on the first flash and 2% on the second flash. These double hits are largely attributable to a small percentage of Q_{400} (oxidized iron) (20), which is a one-time event on the time scale of the flash period. This source of double hits is irrelevant to the comparison of the two S-state cycles discussed here and will be ignored in the following.

Although the oxygen yield pattern is relatively insensitive to the different values of misses for the two cycles at pH values near neutral, significant differences are expected under more extreme conditions, where some of the equilibrium constants become small. For example, Fig. 3 shows the oscillations in oxygen evolution calculated for the transition probabilities expected at pH 8.5, where the electron transfer equilibrium between Q_A^- and Q_B is small [$L_{AB} \simeq 3.5$ (29)].

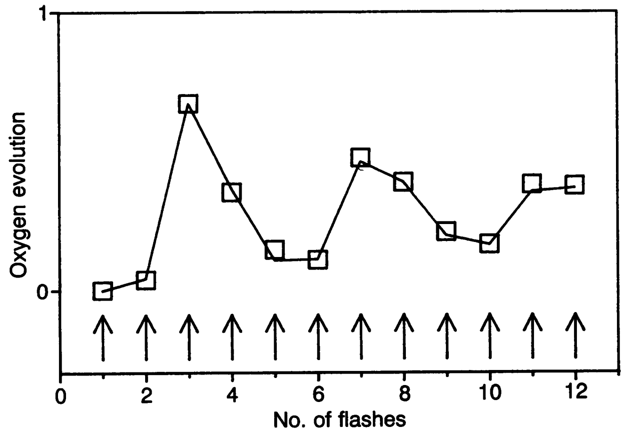

FIG. 2. Flash-induced oxygen evolution calculated from the model with equilibrium constants as for Fig. 1 and 98% flash saturation (solid line). The distribution of the states at the beginning of the flash series was assumed to be $S_0 = 0.25$ and $S_1 = 0.75$. Double hit is 0.1 for the first flash and 0.02 for the second. Experimental data on oxygen evolution from ref. 10 are shown by squares.

In addition to predicting differences in the observed oscillating pattern, the model makes the important, conceptual point of recognizing two parallel S-state cycles and provides a natural way to interpret and estimate misses through the equilibrium constants on the donor and acceptor sides, which can be determined independently. The necessary conclusion is that misses are different for each flash and even depend on the initial conditions—e.g., in which cycle the RC was located before the flash series.

Delayed Fluorescence. At times shorter than a few hundred microseconds after a flash, production of delayed fluorescence is dominated by a fraction of RCs that are inactive in O_2 evolution (26). At longer times, however, the intensity of the delayed fluorescence is proportional to the active population of RCs with oxidized P680 and reduced Q_A (35):

$$L \propto [P^+Q_A^-] \propto [P^+][Q_A^-]).$$

Unlike oxygen evolution, which accompanies only one transition ($D^{3+} \rightarrow D^0$), the intensity of delayed luminescence is an indicator of each transition and shows extreme sensitivity to the type of kinetic cycle. Fig. 4 shows the pattern of oxygen evolution and delayed luminescence precursor ($P^+Q_A^-$), both calculated for cycles V (dashed line) and W (solid line) for the same set of parameters. To show the differences most clearly, they are drawn in the same phase with respect to oxygen evolution. It is evident that, even under conditions where the difference in the oxygen evolution of the two cycles is minimal (Fig. 4*A*), the flash dependence of the intensity of delayed fluorescence exhibits completely different behavior (Fig. 4*B*).

CONCLUSION

Descriptions of oxygen flash-induced patterns and related phenomena of PSII have commonly employed equal miss factors for different flash numbers, although several authors have suggested that they may be different for each S state (9–12). In some cases, 2-fold periodicity of these parameters has been noted (e.g., ref. 12), but the specific association of this behavior with the coupling of the donor and acceptor side reactions has not been made previously. We suggest that the reversibility of the electron transfer processes on the donor

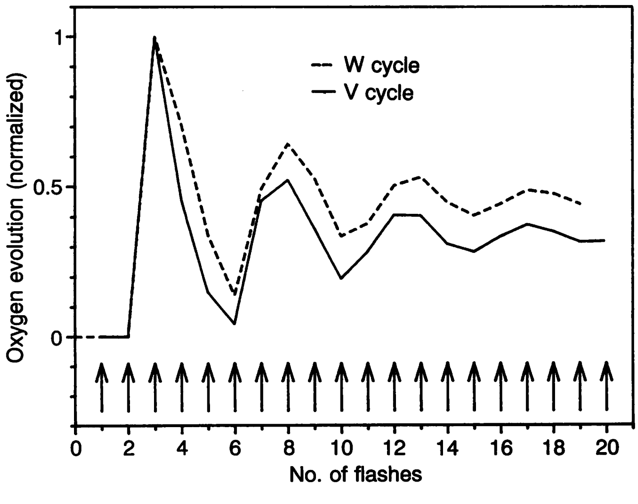

FIG. 3. Calculated flash-induced oxygen evolution in cycle V (solid line) and cycle W (dashed line) with equilibrium constants as in Fig. 1 and $L_{AB} = 3.5$ and 95% flash saturation. For comparison, the two curves are aligned by advancing the W cycle with a one-flash phase shift.

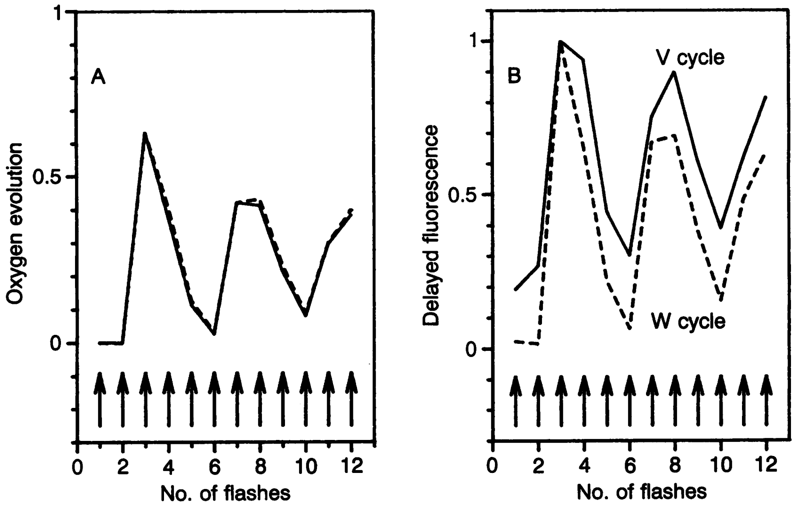

FIG. 4. Flash-induced oxygen evolution (*A*) and delayed fluorescence (*B*) calculated for cycle V (solid line) and cycle W (dashed line) for the set of equilibrium constants as in Fig. 1 with $L_{AB} = 18$ and 95% flash saturation. The two cycles are aligned by a one-flash phase shift advancing cycle W.

and acceptor sides is the main factor leading to damping of the period four oscillation in the oxygen evolution. The model makes explicit the conclusion of Joliot and Kok (3) concerning the transitions on the donor and acceptor sides in oxygen evolution. Thus, the formal misses introduced by Kok *et al.* (4), for saturating flashes, are interpreted as the probability to find RCs in the state either with oxidized P680 or with reduced Q_A. This effect is largely responsible for the damping of period four oscillations in oxygen evolution. Because of the different equilibrium constants involved in different states of the donor and acceptor complexes, the misses calculated from this assumption are different for different states of the RC. Conversely, the miss factor becomes a source of information on the S-state-dependent electron transfer equilibria. Combining the period two cycle of the acceptor reactions with the period four cycle on the donor side produces two distinct cycles, each with periodicity of four but with different values of misses. The binary oscillations of the secondary quinone acceptor in these cycles also have different damping parameters. Hence, even the simplest kinetic model considered here implies an inherent heterogeneity in the flash-induced pattern of PSII RCs.

1. Crofts, A. R. & Wraight, C. A. (1983) *Biochim. Biophys. Acta* **726,** 149–185.
2. Wraight, C. A. (1982) in *Function of Quinones in Energy Conserving Systems*, ed. Trumpower, B. L. (Academic, New York), pp. 181–197.
3. Joliot, P. & Kok, B. (1975) in *Bioenergetics of Photosynthesis*, ed. Govindjee (Academic, New York), pp. 388–413.
4. Kok, B., Forbush, B. & McGloin, A. (1970) *Photochem. Photobiol.* **11,** 457–475.
5. Andersson, B. & Styring, S. (1991) *Curr. Top. Bioenerg.* **16,** 1–81.
6. Vermaas, W. F. J. & Ikeuchi, M. (1991) in *Cell Culture and Somatic Cell Genetics of Plants*, eds. Bogorad, L. & Vasil, I. K. (Academic, San Diego), Vol. 1, pp. 25–111.
7. Witt, H. T. (1991) *Photosynth. Res.* **29,** 55–77.
8. Rutherford, A. W., Zimmermann, J.-L. & Boussac, A. (1992) *Top. Photosynth.* **11,** 179–229.
9. Bouges-Bocquet, B. (1980) *Biochim. Biophys. Acta* **594,** 85–103.
10. Forbush, B., Kok, B. & McGloin, M. (1971) *Photochem. Photobiol.* **14,** 307–321.
11. Delrieu, M.-J. (1983) *Z. Naturforsch. C: Biosci.* **38,** 247–258.
12. Naber, D. (1989) Ph.D. thesis (Univ. Wageningen, The Netherlands).
13. Delrieu, M.-J. & Rosengard, F. (1991) *Biochim. Biophys. Acta* **1057,** 78–88.
14. Melis, A. (1991) *Biochim. Biophys. Acta* **1058,** 87–106.
15. Ort, D. & Whitmarsh, J. (1990) *Photosynth. Res.* **23,** 101–104.
16. Debus, R. J., Barry, B. A., Babcock, G. T. & McIntosh, L. (1988) *Biochemistry* **27,** 9071–9074.
17. Metz, J. G., Nixon, P. J., Brudvig, G. W. & Diner, B. A. (1989) *Biochemistry* **28,** 6960–6969.
18. Debus, R. J., Barry, B. A., Babcock, G. T. & McIntosh, L. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 427–430.
19. Vermaas, W. F. J., Rutherford, A. W. & Hansson, O. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 8477–8481.
20. Diner, B. A. & Petrouleas, V. (1987) *Biochim. Biophys. Acta* **895,** 107–125.
21. Jursinic, P. (1981) *Biochim. Biophys. Acta* **635,** 38–52.
22. Vos, M. H., van Gorkom, H. J. & van Leeuwen, P. J. (1991) *Biochim. Biophys. Acta* **1056,** 27–39.
23. Gerken, S., Dekker, J. P., Schlodder, E. & Witt, H. T. (1989) *Biochim. Biophys. Acta* **977,** 52–61.
24. Buser, C. A., Thompson, L. K., Diner, B. A. & Brudvig, G. W. (1990) *Biochemistry* **29,** 8977–8985.
25. Buttner, W. J. & Babcock, G. T. (1984) *Biochim. Biophys. Acta* **767,** 272–287.
26. Brettel, K., Schlodder, E. & Witt, H. T. (1984) *Biochim. Biophys. Acta* **766,** 403–415.
27. Robinson, H. & Crofts, A. R. (1987) in *Progress in Photosynthesis Research*, ed. Biggins, J. (Nijhoff, Dordrecht, The Netherlands), Vol. 2, pp. 429–432.
28. Zankel, K. L. (1973) *Biochim. Biophys. Acta* **325,** 138–148.
29. Robinson, H. & Crofts, A. R. (1984) in *Advances of Photosynthesis Research*, ed. Sybesma, C. (Nijhoff/Junk, Dordrecht, The Netherlands), Vol. 1, pp. 477–480.
30. Meyer, B., Schlodder, E., Dekker, J. P. & Witt, H. T. (1989) *Biochim. Biophys. Acta* **974,** 36–43.
31. Jahns, P., Lavergne, J., Rappaport, F. & Junge, W. (1991) *Biochim. Biophys. Acta* **1057,** 313–319.
32. Vermaas, W. F. J., Renger, G. & Dohnt, A. (1984) *Biochim. Biophys. Acta* **764,** 194–202.
33. Shinkarev, V. P. & Wraight, C. A. (1992) in *The Photosynthetic Reaction Center*, eds. Deisenhofer, J. & Norris, J. R. (Academic, New York), in press.
34. Styring, S. & Rutherford, A. W. (1987) *Biochemistry* **26,** 2401–2405.
35. Lavorel, J., Lavergne, J. & Etienne, A.-L. (1982) *Photobiochem. Photobiophys.* **3,** 287–314.