A Three-State Model for Energy Trapping and Chlorophyll Fluorescence in Photosystem II Incorporating Radical Pair Recombination

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ABSTRACT The multiphasic fluorescence induction kinetics upon a high intensity light pulse have been measured and analyzed at a time resolution of 10 μs in intact leaves of *Peperomia metallica* and *Chenopodium album* and in chloroplasts isolated from the latter. Current theories and models on the relation between chlorophyll fluorescence yield and primary photochemistry in photosystem II (PSII) are inadequate to describe changes in the initial phase of fluorescence induction and in the dark fluorescence level F_0 caused by pre-energization of the system with single turnover excitation(s). A novel model is presented, which gives a quantitative relation between the efficiencies of primary photochemistry, energy trapping, and radical pair recombination in PSII. The model takes into account that at least two turnovers are required for stationary closure of a reaction center. An open reaction center is transferred with high efficiency into its semiclosed (-open) state. This state is characterized by Q_A and P680 in the fully reduced state and a lifetime equal to the inverse of the rate constant of Q_A^- oxidation (approx. 250 μs). The fluorescence yield of the system with 100% of the centers in the semiclosed state is 50% of the maximal yield with all centers in the closed state at fluorescence level F_m. A situation with ~100% of the centers in the semiclosed state is reached after a single turnover excitation in the presence of 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU). The lifetime of this state under these conditions is ~10 s. Closure of a semiclosed (-open) center occurs with low efficiency in a second turnover. The low(er) efficiency is caused by the rate of P^+ reduction by the secondary donor Y_Z being competitive with the rate of radical pair recombination in second and following turnovers. The single-turnover-induced alterations in the initial kinetics of the fluorescence concomitantly with a 15–25% increase in F_o can be simulated with the present so called three-state model of energy trapping. The experimental data suggest evidence for an electrostatic effect of local charges in the vicinity of the reaction center affecting the rate of radical pair recombination in the reaction center.

INTRODUCTION

The chlorophyll (Chl) *a* fluorescence yield (Φ_F) in green cells and chloroplasts changes substantially with time upon actinic illumination. The time pattern of the light-induced change in fluorescence yield is known as the fluorescence induction curve (Govindjee et al., 1986). In general, it shows a multiphasic rise in high intensity light from an initial low level, called the dark fluorescence yield, toward a 5–7 times higher quasistationary maximal level, reached after ~1 s. In prolonged illumination, the fluorescence yield after 1 s decreases toward a lower level. Ample evidence has been presented that the variable fluorescence comes from photosystem II (PSII). Since its demonstration (Duysens and Sweers, 1963) the variable Φ_F of PSII has been the subject of many reviews (Krause and Weiss, 1991; Dau, 1994; Lazar, 1999). Several models have been presented that quantitate the increase in Φ_F with the decrease in the efficiency of photochemical energy conversion (Φ_p) in the photosynthetic reaction centers (RC) due to their closure. Closure of an RC finishes its capability for energy trapping. In bacteria, the complementary relation between Φ_F and Φ_p was demonstrated for the first time (Vredenberg and Duysens, 1963). In PSII, the light-driven reduction of the primary quinone electron acceptor (Q_A) is considered to reflect the closure of the RC; this reduction is thought to release the quenching properties of the oxidized form. Fluorescence changes elicited with (sub)nanosecond excitations have indicated that the oxidized primary donor of PSII ($P680^+$) quenches the fluorescence as well (Butler, 1972; Mauzerall, 1972).

Measurement of fluorescence induction has now become a routine method in photosynthetic research. The availability of quantitative models and detection methods with improved sensitivity and time resolution has greatly contributed to the application of this noninvasive fluorescence scanning method in photosynthesis research in a broad sense (Schreiber et al., 1986; Strasser et al., 1995). However the time pattern of the fluorescence induction in the time range between 10 μs and 1 ms shows a behavior in response to energization with single turnover excitation that cannot easily be interpreted with existing descriptive models. This paper deals with the inadequacy of the current models to relate changes in the fluorescence yield with data on RC-generated photocurrents. A new model relating energy trapping and fluorescence will be presented.

In addition to fluorescence and other techniques (see Amesz and Hoff, 1996) the bioenergetic performance and behavior of the photosynthetic (thylakoid) membrane can be studied in intact cells and chloroplasts with electrophysiological methods (Vredenberg, 1997; Bulychev and Vreden-

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berg, 1999) using, among others, conventional patch clamp suction electrodes. It has been demonstrated that, by applying single-turnover (<6 μs) saturating light flashes to a patched chloroplast in a so-called whole thylakoid configuration, the operation, number, and actual performance of the two light-dependent current generators can be quantified from the photocurrent (-potential) responses (van Voorthuysen et al., 1996; van Voorthuysen, 1997). The multiphasic photocurrent profiles upon a train of multiple single turnover flashes (1 s^{-1}) show the following characteristics (Vredenberg et al., 1998a): 1) photocurrent generation originates from transthylakoid charge transfer (separation) accompanying RC- and Q-cycle turnover, 2) no decline in the number of transfer-competent (active) RCs in a flash train within a 3% detection limit, 3) a binary oscillation of the Q-cycle current generator with high activity in even-numbered flashes, 4) a 15–30% decrease in the amplitude of the RC-driven current in the second and following flashes of a flash train concomitantly with an increase in the dark recovery time of the photocurrent response. The decrease in amplitude and decay rate constant of the photocurrent in a double flash after dark adaptation has been interpreted in terms of a decrease in the electric conductance of the thylakoid lumen. It has been presumed that the single-flash-saturated decrease in the electrical lumen conductance, which occurs at a rate of about 100 s^{-1}, originates mostly, if not completely from a change in thylakoid geometry, notably a contraction of the thylakoid system leading to a narrowing of lumenal sheets (Vredenberg et al., 1998a). The effect of this low light requiring change in thylakoid configuration is enhanced by its low dark relaxation rate of less than 10^{-2} s^{-1}.

In searching for bioenergetic parameters with comparable low light requirement and dark relaxation that might be related with dynamic changes in thylakoid configuration, we focussed upon changes in chlorophyll-*a* fluorescence yield, in particular on studies in which the light-on fluorescence induction phase has been studied in dependence of preactivating single-turnover flashes (Delosme, 1971; Schreiber and Neubauer, 1987; Strasser and Strasser, 1998). The characteristics of the flash-dependent changes in the dark fluorescence yield $[\Phi_F]_0$ of isolated chloroplasts show similarities with those of the changes in lumen conductance. $[\Phi_F]_0$ is increased by 10–20% by one single flash; this increase shows a four-periodic modulation in response to the number of flashes with a maximal $\Delta[\Phi_F]_0$ of 20–25% at the 2nd, 6th etc flash and minima of 5–15% at the 4th, 8th etc flash (Schreiber and Neubauer, 1987; Strasser and Strasser, 1998). The single-flash-saturated increase in $[\Phi_F]_0$ of similar size as in chloroplasts was also found to occur in intact leaves with a dark relaxation rate of about 10^{-2} s^{-1} (Vredenberg et al., 1998; G. Rodrigues, personal communication).

The four-periodic modulation of flash-induced $\Delta[\Phi_F]_0$ points to a relation with the (S) state of the oxygen evolving complex (OEC). Recently, mechanisms and quantitative models of the variable PSII fluorescence have been discussed and presented in many papers (Trissl et al., 1993; Dau, 1994; Schreiber and Krieger, 1996; Stirbet et al., 1998; Bernhard and Trissl, 1999; Lazar, 1999). However none of them is adequate to give a quantitative description of the light effect on F_o and on the initial phase of the fluorescence induction pattern. A nomenclature has been introduced (Strasser et al., 1995) for the multiphasic rise from $[\Phi_F]_0$ at level O to $[\Phi_F]_M$ at level P via two intermediate levels J- and I. Accordingly the rise is denoted by the so-called O-J-D-I-P rise. We refer herein to the O-I_1-D-I_2-P nomenclature introduced by Schreiber (Schreiber and Neubauer, 1987), which accounts for the occurrence of a dip (D) between the I_1 (= J) and I_2 (= I) level in high intensity light.

In this paper, we present a new energy trapping model of PSII that emphasizes that closing of an RC requires two successive trapping events. Expressions are given that relate the fluorescence yield during the 0.01–10-ms induction phase at the O-, J- and I- level, i.e., $[\Phi_F]_0$, $[\Phi_F]_J$ and $[\Phi_F]_I$, respectively, with the photochemical yield of PSII (denoted as Φ_p) and the yield of radical pair recombination in the RC (Φ_{rp}). The model takes into account an electrostatic effect of local charge densities in the vicinity of the RC on the rate of radical pair recombination (k_{-1}). Electric field-dependent changes in k_{-1} show up as changes in Φ_{rp} and consequently as changes in $[\Phi_F]_0$ and $[\Phi_F]_J$. A simulation of the flash-dependent kinetics of the O-J rise in the fluorescence induction trace in strong actinic light is also shown. Its modification by a single flash preillumination is presumed to be caused by changes in Φ_{rp}.

MATERIAL AND METHODS

The fluorescence experiments were done with young intact leaves of *Peperomia metallica* plants grown in pots at 12/12 h photoperiod in an atmosphere-controlled light cabinet at 1 W m^{-2} PAR, as described in detail elsewhere (van Voorthuysen, 1997). In some experiments, leaves of *Chenopodium album* L and chloroplasts isolated thereof were used. These were grown (Jansen et al., 1986) at 30 W m^{-2} PAR in a 16/8 h photoperiod. Isolation procedure and media were as described elsewhere (Curwiel and van Rensen, 1993).

Changes in fluorescence emission (yield) were measured with a PAM chlorophyll fluorometer (Heinz Walz, Effeltrich, Germany) and with a Plant Efficiency Analyzer (PEA, Hansatech Instruments, King's Lynn, England). The PAM was used for measuring changes in F_O after a single turnover-saturating flash (Xe lamp, halfwidth < 6 μs). The intensity of the measuring light was maximally attenuated to compromise a high sensitivity and response time for the relatively small changes in F_O with no actinic activity of the measuring light. For this purpose, the measuring light was interrupted during the 15-s dark intervals between the ~1-s periods during which F_O was probed. The PEA was used for data retrieval of the fluorescence induction upon the onset of a 1-s high-intensity red (maximal emission at 650 nm) light pulse (600 W m^{-2} at 100% intensity, which corresponds with an intensity of about 3.5×10^3 μmol·m^{-2}·s^{-1}). With a chlorophyll [chl] content in an average leaf of ~0.6 mmol·m^{-2} (Lawlor, 1993) and an RC density of PSII of ~6×10^2 chl/RC, one turnover of an

RC will take about 100 μs at maximal intensity of the setup. Fluorescence data were collected at an acquisition interval of 10 μs as described in more detail elsewhere (Strasser et al., 1995). The F_O level was taken as the extrapolated signal at 10 μs after the onset of the illumination as is illustrated in Fig. 1. This was done by extrapolation of the log plot of the first derivative of the response in the 50–150-μs time domain. In general, this was a linear extrapolation due to an apparent exponential increase of the initial fluorescence. Extrapolation could only have been done under conditions at which the rate constant of the initial fluorescence rise is distinctly smaller than the rate constant of the onset of the fluorescence detector. In the PEA instrument, the latter is about $(30\ \mu s)^{-1}$. With Peperomia leaves, this condition was satisfactorily fulfilled only at an intensity below 450 W m^{-2}, i.e., below an ~75% setting of the maximal intensity.

RESULTS AND MODEL

The left-hand panel of Fig. 2 shows fluorescence (F) induction curves in a Peperomia leaf during 5 ms after the onset of an ~450 W m^{-2} light pulse of 1 s duration. The two curves are from a 30-min dark-adapted leaf that has been given one single turnover flash (sf-) or not (da-). They show clear differences in the F_o-level and in the fluorescence induction during the first ms of the light pulse. The right-hand panel shows the same response plotted on a log-time scale from 10 μs to 1 s. As compared to the da-leaf the sf-leaf is characterized by 1) higher F_o (in this case 22%), 2) an almost monoexponential fluorescence increase in the 10–100-μs rise phase (not explicitly shown, but see Fig. 7), 3) a quasistationary fluorescence level in the 100–500-μs time range, and 4) absence of the transient spike with a clear fluorescence decline in the time interval between 1 and 3 ms, which is seen in the da-leaf. The induction curve is labeled with O-, J-, D-, I-, and P. A Chenopodium leaf shows a similar pattern and behavior (Fig. 3), except for the presence of two bending regions between J (or D) and P. These have been designated with I_a and I_b. Comparable effects have been reported in spinach chloroplasts (Neubauer and Schreiber, 1987) and in leaves (and chloroplasts) from other plant varieties (Strasser and Strasser, 1998). Hitherto, the effects of pre-flashes on F_o and the initial rise kinetics have not been interpreted in relation to each other. As seen in Figs. 2 and 3, the effect of preillumination is mainly in the O-J-D part of the induction curve. The I- and P levels are hardly, if at all, affected by the pre-flash.

Qualitatively, the O-J-D part of the fluorescence in the sf-leaf (Fig. 2) shows that the first turnover after turning on the light, which, at the given excitation rate of 10^4 s^{-1}, takes about 100 μs, leads to a rise in the fluorescence level from $F_o = 1.22$ to $F \sim 3.8$. This level hardly changes, if at all, in the next 2–4 turnovers in the 100–500-μs time range. A further rise to the I-level at $F \sim 5.7$ occurs in the 1–10 ms range. Conversely, in the da-leaf, the fluorescence continuously rises during the first 5–10 turnovers in the 0.01–1-ms time range to a J-level at $F \sim 5$. This rise is followed in the next 1–3 ms by a small decrease to a D-level, after which a rise to the I- and P-level at $F \sim 7.5$ starts following a similar pattern as in the sf-leaf. These data would be in harmony with a reaction scheme which predicts that the RC of a dark-adapted system in a first turnover of a multiturnover light pulse are transformed into a quasistationary state, which, further, can be transferred by subsequent turnovers in two steps, the first of which is substantially retarded by a pre-flash. It should be remarked that the fluorescence induction pattern of the da-leaf in a 1-s multiturnover light pulse as used here is likely to be affected by the light pulse, independent of the reaction pattern that underlies the fluorescence increase. This is because this pulse, like the effect of a single turnover of a Xe flash, will cause a change in the reaction pattern of the fluorescence rise in a dark-adapted system. We presume that the interference of this effect is in the time range beyond 10 ms.

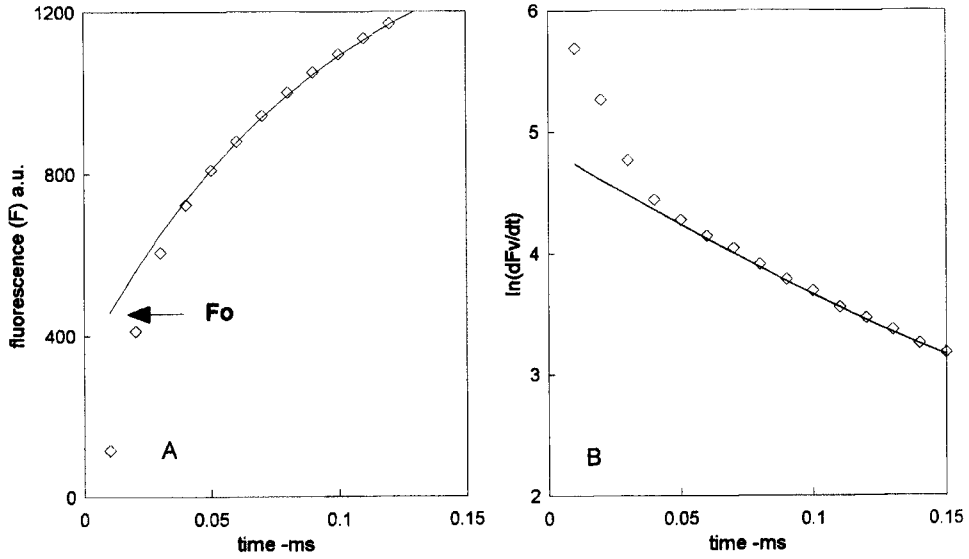

FIGURE 1 Fluorescence (F) data points of a dark-adapted Peperomia leaf collected in a Plant Efficiency Analyzer during the first 0.15 ms of illumination with 450 W m^{-2} light (*open symbols* in *A*) and a log plot of the first derivative of these data during the first ms of illumination (*open symbols* in *B*). Extrapolation of the slope in *B* in the time region 0.05–0.15 ms to the time domain <0.05 ms, where the fluorescence detector is limiting with a response time of ~0.03 ms, gives the fluorescence emission data in the 0.01–0.05-ms time domain (*solid curves* in *A* and *B*).

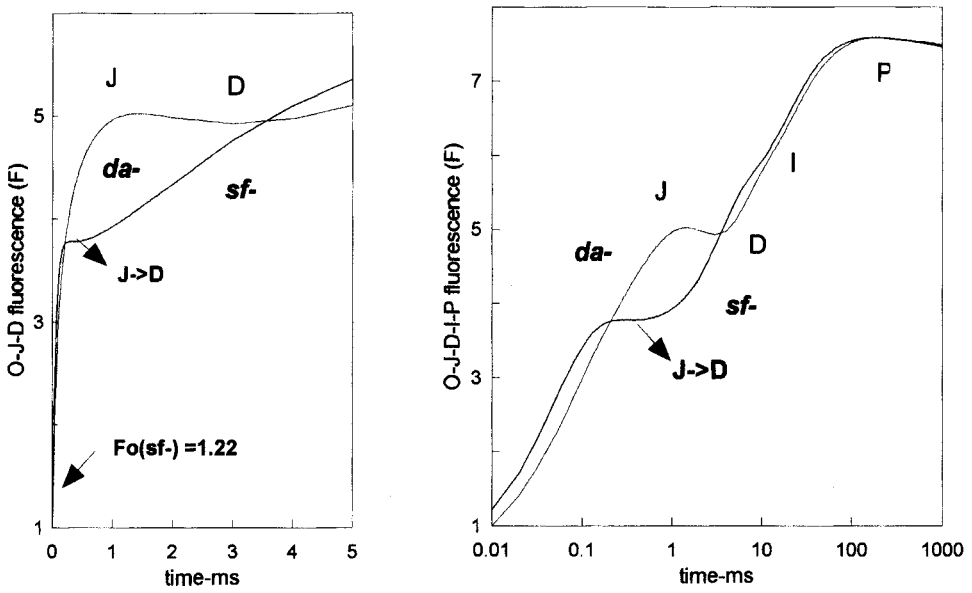

FIGURE 2 Fluorescence induction kinetics of a dark-adapted Peperomia metallica leaf before (da-) and 5 s after a single turnover pre-flash (sf-) plotted on a linear (*left panel*, but only for the first 5 ms) and a log time scale (*right panel*) with notations for the O-J-D-I- and P- levels. The curves have been normalized on the F_o level ($F_o = 1$) of the da-leaf. Note the difference in F_O and the different kinetics during the first 10 ms for the sf-leaf. Intensity of light was 450 W m^{-2}. Noteworthy in the pre-flashed leaf is the ~22% higher F_0, the absence of the transient J-D phase of the fluorescence induction, the nearly constant fluorescence yield at the J level in the 0.1–1-ms time interval with $F_v/F \sim 0.7$, and coincidence with the da-curve above $t \sim 20$ *ms*.

Figure 4 shows the initial fluorescence F_o measured before and at various times after a single turnover light flash in a dark-adapted Peperomia leaf. It shows for this leaf an ~18% increase in F_o and a recovery of this single-turnover effect with a rate constant of ~100 s^{-1}. Companion photocurrent measurements with patched chloroplasts from the same or a similar dark-adapted leaf (data not shown, but see Vredenberg et al., 1998a,b) have suggested evidence that a

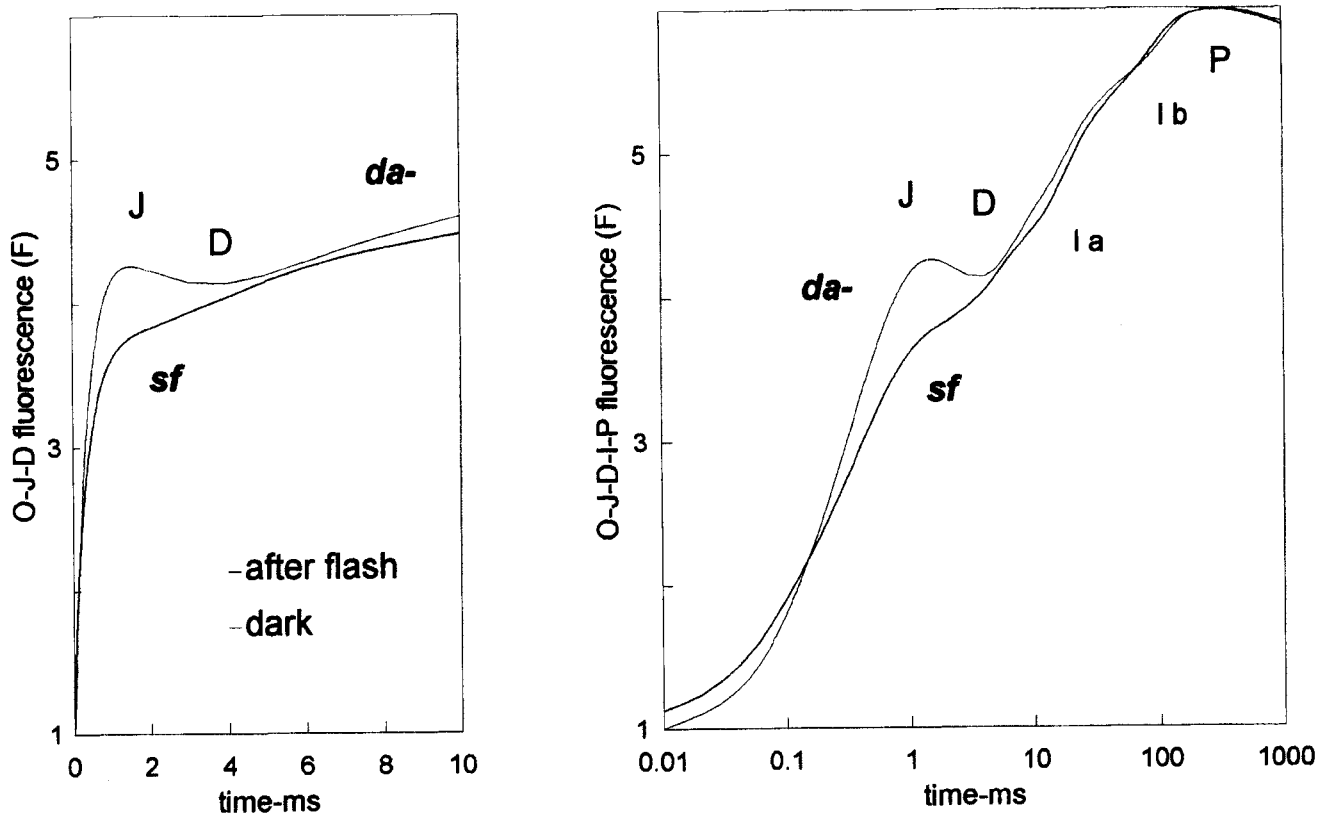

FIGURE 3 Fluorescence induction kinetics of a dark-adapted Chenopodium leaf before (da-) and 5 s after a single turnover saturating Xe flash (sf-). The curves have been normalized on the F_o level ($F_o = 1$) of the da-leaf. Note the difference in F_O and the different kinetics during the first 10 ms for the sf-leaf. Intensity of light was 600 W m^{-2}. There is an intermediate hump in the J-I phase, which has been renamed J-I_a-I_b. See also legend of Fig. 5

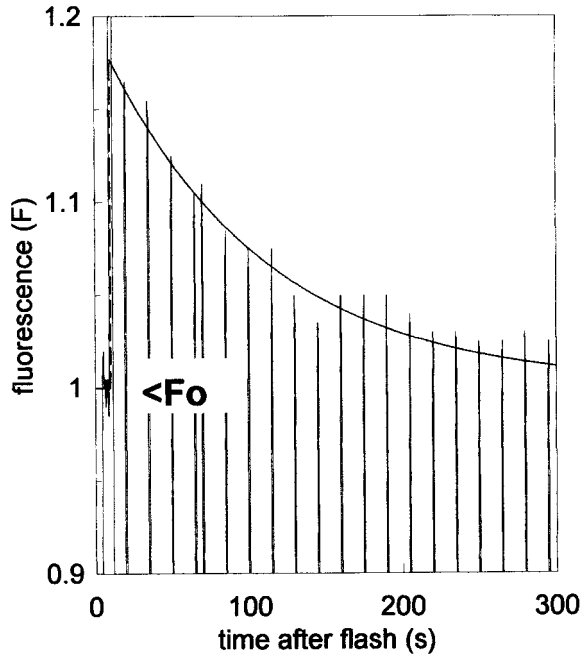

FIGURE 4 Fluorescence (F) yield (upward deflections) in a Peperomia leaf before ($F = F_O = 1$) and after a single turnover saturating pulse measured at 15-s intervals by low modulated light in a PAM fluorometer. The modulated excitation light beam was switched off for ~12 s every 15 s. The dashed vertical line marks the time at which the single turnover Xe flash was fired. The continuous curve is the exponential fit of $\Delta F/F_O$ (17.8%) with a relaxation time of 105 s.

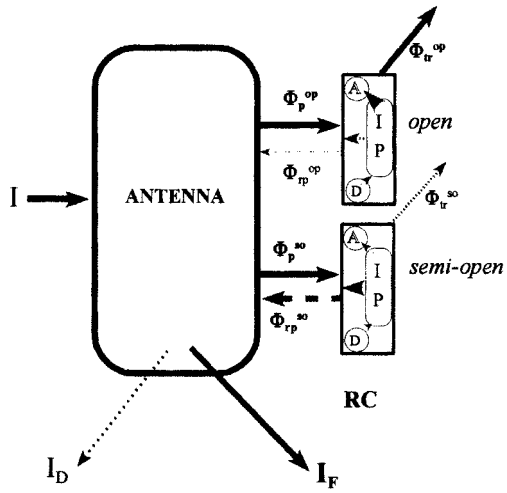

FIGURE 5 Energy trapping and fluorescence in PSII. I is the incident energy flux; Φ_p, Φ_{rp}, and Φ_{tr} refer to probabilities for photochemical charge separation, radical pair recombination, and photochemistry (Q_A reduction), respectively, with superscripts o and so referring to the open and semiopen state, respectively. The thickness of the arrows for Φ_{tr} and Φ_{rp} (*dashed*) is indicative for their respective magnitudes. Nonradiative (heat) losses in the reaction center are assumed to be relatively small in comparison with radical pair recombination and photochemistry The diagonal oriented arrow points symbolize electron transfer at donor and acceptor side; radical pair recombination is symbolized with the horizontal arrow in the middle. Further explanations are in the text.

single turnover light flash is required and sufficient to cause a contraction of saturated size of the thylakoid lumen. The time constant of recovery of this response observed as a change in the lateral resistance of the internal chloroplast compartments has been measured (Vredenberg, 1998b) and is of the same order of magnitude as the F_o recovery shown in Fig. 4. In addition, these patch clamp experiments indicated that the number of excitable RCs is the same in a second and following turnovers in a train of saturating single-turnover flashes.

A reaction mechanism that would explain the changes in the fluorescence parameters of a dark-adapted system in response to a single pre-flash as illustrated in Figures 2–4 cannot be derived from the current models of fluorescence (induction) in relation to energy trapping. Figure 5 gives an illustration of a new model which will do so for an individual photosynthetic unit (PSU). A brief outline of this so-called three-state model of energy trapping in PSII, which discriminates between open, semiopen (-closed), and closed centers is given below.

States of the reaction center; definitions

The PSII D_1/D_2 RC dimer is considered here as the monomer with P680 (P) and Pheophytin (I) as the primary electron donor and acceptor in the photochemical charge separation, respectively (Klimov and Krasnovskii, 1981). A redox active tyrosin, usually denoted as Y_Z, and a bound plastoquinone Q_A are the secondary donor and acceptor, respectively. As a convenient short-hand notation, we will denote Y_Z and Q_A here as D and A, respectively. At the donor side of the RC, electrons are supplied to D via the oxygen evolving complex (OEC). The OEC cycles, via 4 one-electron oxidation steps through its S states, starting in the dark from $S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_0 \rightarrow S_1$. Ample evidence has been given that the electron transfer rate at the donor side depends on the S-state, presumably governed by electrostatic effects (Dekker et al., 1984; Dau, 1994). At the acceptor side, electrons are delivered to the cytochrome b/f complex via a two-electron reduction of a second plastoquinone (Q_B), which exchanges with the plastoquinone pool (Velthuys and Amesz, 1974; Robinson and Crofts, 1983).

We will consider the condition in which 1) the electron efflux from the RC at the acceptor side, which is determined by the rate of A^- (Q_A^-) oxidation (k_{-q}), is comparatively low in relation to a high excitation rate (influx), and 2) each RC functions with a partial connectivity of associated antennae with neighboring ones (connected unit model, see Joliot and Joliot, 1964; Strasser, 1981). The connectivity and its effect on the kinetics of the state transfer of an RC can be quantified with a so-called connectivity coefficient denoted here with p (Strasser, 1981, but there signified with another character). With an estimated value of $k_{-q} = (250\ \mu s)^{-1}$

(Kolber et al., 1998; and see Dau, 1994) and a turnover time of about 100 μs, the first condition is reasonably fulfilled at the high intensities used. On the basis of the data of Fig. 2, the turnover time in a *P. metallica* leaf at the onset of illumination with 400 W m^{-2} is about 50 μs.

The reaction center is called open when A (Q_A) is in the oxidized state and the probability for exciton transfer to it (Φ_p) is different from zero due to the trapping competent state of the primary donor and acceptor pair PI. In this state, charge separation can take place converting PI into the radical pair P^+I^-. The RC is defined to be in a closed state when it is unable to trap singlet energy because either I is in the reduced (I^-) or P in the oxidized form (P^+). A closed state with PI^- is unlikely to exist after a first turnover because, in a dark-adapted system, the rate constants of A-(Q_A^-) reduction (k_q) and of P^+ reduction (k_s) are of the order of 10^9 and 10^7 s^{-1}, respectively, i.e., differ by two orders of magnitude (see Dau, 1994). The intermediate closed state $D(P^+I)A^-$ formed in the first turnover is transient. It is transformed into state $D^+(PI)A^-$ upon reduction of P^+ after electron acceptance from D. This occurs with rate constant k_s. The state $D^+(PI)A^-$ is special in a sense that it can collect only one second electron. This state is called the semiopen (or semiclosed) state. A semiopen state refers to a dynamic state of the RC capable of charge separation and with A (Q_A) in the reduced form. Thus an RC can be in three distinctly different states: open, semiopen (-closed), and closed. These states differ in a way that they are a 2-, 1-, and 0-electron trap, respectively. Because an open and closed state differ by two electrons, it is impossible to transfer an open RC into a stationary closed state by a single turnover. The closed states $D(P^+I^-)A$ and $D(P^+I)A^-$ are called transient closed states because they are sequentially transferred into either a semiopen state, or into an open state by means of charge recombination of the radical pair. However, the probability for the latter conversion (Φ_{rp}) is low for these transient states as we will discuss below. Moreover, the transitions occur in <10 μs, which is below the observation time window considered here. Time analyses of fluorescence induction kinetics in the submicrosecond time domain can be found in other papers (Trissl et al., 1993; Bernhardt and Trissl, 1999).

Exciton transfer, trapping and fluorescence yield

The model (Fig. 5) takes into account and illustrates that exciton transfer from the antenna to an open and semiopen RC occurs with, for each, an equal efficiency (probability) Φ_p. It also takes into account and considers that stationary closure of an RC requires at least two turnovers. The closed RC is not shown, because for this state Φ_p is zero. Back transfer of excitons from open and semiopen RC to antenna occurs with a probability designated with Φ_{rp}^{op} and Φ_{rp}^{so}, respectively. The efficiency of exciton trapping in open and semiopen RCs is designated with Φ_{tr}^{op} and Φ_{tp}^{so}, respectively. For the sake of simplicity, we assume that the fate of an exciton in the RC is either being trapped (Φ_{tr}), or being back transferred to the antenna (Φ_{rp}). This means that other losses are assumed to be comparatively small and that $\Phi_{tr} + \Phi_{rp} = 1$. The collective dissipative energy flux from the antenna, other than the fluorescence (I_F), is designated by I_D. The model refers and is restricted to conditions at which 1) the excitation rate of the PSU exceeds the electron transfer rate at the acceptor side; 2) the PSU is in a dark-adapted state, i.e., with 100% open state and $S = S_1$; and 3) Φ_{rp}^{op} is close to zero, which means nearly 100% trapping efficiency in the RC, i.e., $\Phi_{tr}^{op} \sim 1$ and, consequently, an almost 100% transfer of the open centers into the semiopen state in each first turnover when followed at a time resolution of 10 μs. Thus, with an excitation rate of 10^4 s^{-1} and averaged over an initial illumination period of $\sim$100 μs, during which one turnover has been made, exciton transfer has occurred reaching an approximately equal concentration of open and semiopen (-closed) RCs.

The energy influx (J_i) and efflux (J_e) to and from the antenna with a dark-adapted (S_1) RC in a single turnover can now be written as

$$J_e = I_F + I_D + \Phi_p * I$$

and

$$J_i = I + (\Phi_{rp}^{op} + \Phi_{rp}^{so}) * \Phi_p * I * 0.5.$$

At equilibrium $J_e = J_i$, which gives $I_F = I * [1 - \Phi_p * (1 - \Phi_{rp}/2)] - I_D$ in which $\Phi_{rp} = \Phi^{op}p_{rp} + \Phi_{rp}^{so}$.

Thus, for this initial time period of a first turnover event, the fluorescence yield is given by

$$\Phi_F = I_F/I = 1 - \Phi_p * (1 - \Phi_{rp}/2) - \Phi_D, \qquad (1)$$

in which $\Phi_D = I_D/I$. For ease of presentation, we virtually include Φ_D as a fractional component in Φ_F. This is allowed with the implicit assumption that the rate constant of the dissipative losses (k_D) does not change independently of the other competitive rate constants.

According to Eq.1, the fluorescence yield is dependent on both the efficiency of exciton transfer to and of radical pair recombination in the RC. Thus, it predicts that, under conditions at which Φ_p is constant, a change in Φ_F will occur in association with a change in Φ_{rp}. The experimental data on the dark fluorescence yield ($[\Phi_F]_o$) and the initial OJ rise in a pre-flashed (sf-) leaf (Fig. 2) can easily be interpreted in terms of Eq.1. Based on the fact that the number of active centers is invariable with flash number (Vredenberg et al., 1998b) Φ_p is equal for the dark-adapted (da-) and sf-leaf. The higher value of $[\Phi_F]_o$ in the sf-leaf as compared to the dark yield in the da-leaf is likely to be caused by a higher efficiency of radical pair recombination in open centers Φ_{rp}^{op} (Eq.1 and Fig. 5) of the sf-leaf. Eq. 1 yields that, at the onset

of excitation with 100% open centers and with $\Phi_{rp}^{op} \sim 0$,

$$\Phi_F = [\Phi_F]_o \sim 1 - \Phi_p. \quad (1a)$$

If we assume that the increase in $[\Phi_F]_o$ is not contaminated with an altered contribution of a low PS1 emission component, then the difference in Φ_{rp} responsible for the 22% increase in $[\Phi_F]_o$ (Fig. 2) can be quantified from Eq. 1. If we take $\Delta[\Phi_F]_0/[\Phi_F]_0 = \delta$ then, with Φ_p assumed to be constant, it easily follows that

$$\Delta\Phi_{rp} = 2\delta(1 - \Phi_p)/\Phi_p + \delta\Phi_{rp}. \quad (2)$$

If one takes for dark-adapted intact chloroplasts and leaves an average value of $\Phi_p = 0.8$, we get with $\delta = 0.22$ (Fig. 2) $\Delta\Phi_{rp} = 0.11 + 0.25\Phi_{rp}$. This means that, in comparison with a dark-adapted open state where $\Phi_{rp}^{op} \ll 1$, the probability for radical pair recombination in a dark-adapted system that has been preilluminated with one saturating flash is at least 0.11. Moreover, as $\Delta[\Phi_F]_0/[\Phi_F]_0$ relaxes in minutes (Fig. 4), the increase in Φ_{rp} persists for several tens of seconds. Because $\Phi_{tr} + \Phi_{rp} = 1$, the increase in Φ_{rp} has, of course, caused an identical decrease in the trapping probability Φ_{tr}. This means that, for the experiment illustrated in Fig. 5, the efficiency of exciton trapping in the pre-flashed (sf-)leaf in a first turnover, i.e., during the first 100 μs, is 89% of the rate of trapping in the dark-adapted (da-) leaf. The experimental curves indicate an approximately equal efficiency. It might be, as we will discuss below, that the ~10% decrease in trapping efficiency associated with the increase in Φ_{rp} is compensated by an increase in the antenna size. The quasi stationary fluorescence level reached after 100 μs suggests that a transfer of the open centers into their semiopen state has been completed and that the efficiency of exciton trapping in these centers (Φ_{tr}^{so}) during subsequent turnovers in the 100–500-μs time range is low, or, in other words, that the efficiency of radical pair recombination therein (Φ_{rp}^{so}) is high.

The efficiency of charge recombination of the radical pair $P^+ I^-$ is determined by the rate constant of this recombination (k_{-1}) and that of the rates of electron donation to P^+ and A (Q_A) at the donor and acceptor side of the RC, respectively (Fig. 5). Because the rate constant of electron transfer to A (k_q) in a first flash with all centers in the open state is about 3×10^9 s^{-1} (Schatz et al., 1988; Roelofs et al., 1992; Dau and Sauer, 1992; Dau, 1994) and (with S = S_2, due to the pre-flash) to that of P^+ (k_s) is about 5×10^7 s^{-1} (Meyer et al., 1989; Dau, 1994), Φ_{rp} in a first flash will be determined mainly by k_{-1} and k_q. With Φ_{rp}^{op} ($= k_{-1}/(k_{-1} + k_q)$) = 0.11 and $k_q = 3 \times 10^9$ one gets, in fair agreement with reported values (Roelofs et al., 1992) $k_{-1} = 3.7 \times 10^8$ s^{-1}. In second and following turnovers starting with nearly 100% of the RCs in the semiclosed state with A reduced (Q_A^-) the actual electron transfer rate to A is determined by the rate constant of Q_B reduction (k_{-q}) and that of subsequent reduction steps in the electron transport chain at the acceptor side and will slow down beyond k_{-q}, which is ~$(250\ \mu s)^{-1}$. Similarly, the actual rate of P^+ reduction in the second and following turnovers becomes increasingly determined by the rate constant of the reduction of the oxidized donor D by S_3 and by following S-states. It will oscillate around an average value of $k_s \sim (500\ \mu s)^{-1}$ (for data see Dau, 1994). Because the excitation rate (~$100\ \mu s)^{-1}$ is not far below k_s, the reduction rate of P^+ in the first turnovers is largely determined by the rate of D-oxidation, which has been reported to be of the order of $(100\ ns)^{-1}$. Thus, for the next coming turnovers with $k_{-q} \leq 4 \times 10^3$ s^{-1}, $k_s \sim 10^7$ and with $k_{-1} = 3.7 \times 10^8$ s^{-1}, $\Phi_{rp}^{so} \sim 0.97$ and Φ_{tr}^{so} is ~3%. Thus, in the sf-leaf, due to the comparatively high value of k_{-1}, i.e., with Φ_{rp}^{so} approaching 1, the efficiency of exciton trapping in semiopen (-closed) centers is low. This low efficiency is conclusive with the quasistationary fluorescence level in the 100–500-μs time range in Fig. 2. With $\Phi_{rp}^{so} \sim 1$ for the semiclosed state, the fluorescence yield of this state, according to Eq.1, is given by

$$\Phi_F = [\Phi_F]_{so} = 1 - \Phi_p/2. \quad (1b)$$

The quasisteady-state fluorescence yield at the J→D level $F_{J(D)}$ is not necessarily identical with $[\Phi_F]_{so}$ because of 1) contributions of turnovers that have caused a transient closure and subsequent back transfer to semiopen centers or 2) back transfer of the semiopen state to the closed state due to reoxidation of A^- (Q_A^-) by Q_B. The first may, for instance, have occurred in the second and third turnover, at which Φ_{rp}^{so} may still be appreciably less than 1 because of the rate of P^+ reduction (k_S) is not yet determined by the rate of D^+ reduction and still of the order of 10^8 s^{-1} as in the first turnover. As we will discuss below, the numerical fit of the experimental fluorescence data of the sf-leaf (Fig. 2) suggest that this is the case in the second turnover. With respect to the second possibility, it can easily be derived that the relation for the fluorescence yield of the semiopen state ($[\Phi_F]_{so}$) is modified into

$$\Phi_F = [\Phi_F]_{so} = 1 - \Phi_p(1 - \beta/2), \quad (1c)$$

when the fraction of RCs that can become semiclosed in a first turnover (β) is less than 1. If $\beta < 1$, $[\Phi_F]_{so} < 1 - \Phi_p/2$. This, for instance, will be the case if the rate of Q_A^- oxidation cannot be neglected during the turnover, even at the maximal light intensity. This, for instance, is the case when the excitation rate is not sufficiently below k_{-q}.

The closure of the semiopen centers with $[\Phi_F]_{so} \sim 1 - \Phi_p/2$ is due to the decrease in Φ_p and occurs with a relative low rate as a consequence of the low efficiency of exciton trapping in the semi-closed centers. According to Eq.1, the fluorescence yield of the system with 100% of the centers closed ($\Phi_p = 0$) is

$$[\Phi_F]_{cl} = 1. \quad (1d)$$

The fluorescence rise in the sf-leaf (Fig. 2) in the 1–10-ms time range from the J- to the I-level at F ~ 5.7 is presumed to be the reflection of the full closure of the semiopen reaction centers. It is the final step in RC closure in association with exciton trapping.

Simulations of the O-J-D-I fluorescence rise

Figure 6 shows a quantitative simulation of the fluorescence increase during the subsequent O-J-D- and J-D-I phases in the pre-flashed Peperomia leaf. The O-J-D phase of the fluorescence rise can be fitted with the sum of two functions, F_1 and F_2. Each of these is the product of two exponential functions and are simulations of the semi (F_1) and full (F_2) closures of the open and semiopen RC in the first and second turnover, respectively.

$$F_1 = b_1 * [1 - \exp(-k_{o\text{-}j}t)] * [\beta + (1 - \beta) * (1 - \exp(-k_{-q}t))], \quad (3)$$

in which $b_1 = [\Phi_F]_{so} - [\Phi_F]_o$ and β is equal to the fraction of centers that has become (semi)closed in the first turnover; k_{oj} is the rate constant of the fluorescence rise associated with the semiclosure of the RC, k_{-q} is related, but not necessarily identical with, the rate constant of Q_B reduction (i.e., Q_A^--oxidation).

$$F_2 = \beta(t) * b_2 * [1 - \exp(-k_{o\text{-}j\text{-}d}t)] * \exp(-k_2 t), \quad (4)$$

in which $\beta(t)$ is the fraction of semiclosed centers at time t, $b_2 = [\Phi_F]_{cl} - [\Phi_F]_{so}$, k_{odj} is the rate constant of the closure of a semiopen RC. The reversion of a closed center into a semiopen center is approximated with the relaxation constant k_2. This back transition is caused by the decrease in Φ_{tr}^{so}, i.e., in $k_{o\text{-}j\text{-}d}$, due to, as outlined before, the decrease in k_s with turnover number and by the rate of Q_B turnover, which, in ongoing turnovers, becomes limited at its oxidation side. The exponential rise functions $[1 - \exp(-kt)]$ in F_1 and F_2 can be transferred into an algorithm by introducing for each of them the connectivity parameter p to accommodate the experimental data of the O-J-D rise. With $p = 0$ (i.e., separate units) the algorithm is identical with the exponential function. In Table 1, the algorithm is characterized by both parameters. The J-I and I-P rise can be fitted with an algorithm derived from the function $b * [1 - \exp(-kt)]$ and a connectivity parameter in which the amplitude b represents the difference between the fluorescence yields at two subsequent levels, and k is the rate constant fitting the experimental rise. The pertinent values of the fit parameters are given in Table 1. As is shown for the pre-flashed (sf-) Peperomia leaf (see insert in right-hand panel of Fig. 6) the simulated curve in the 0.01–5-ms time interval deviates less than 1% from the experimental response.

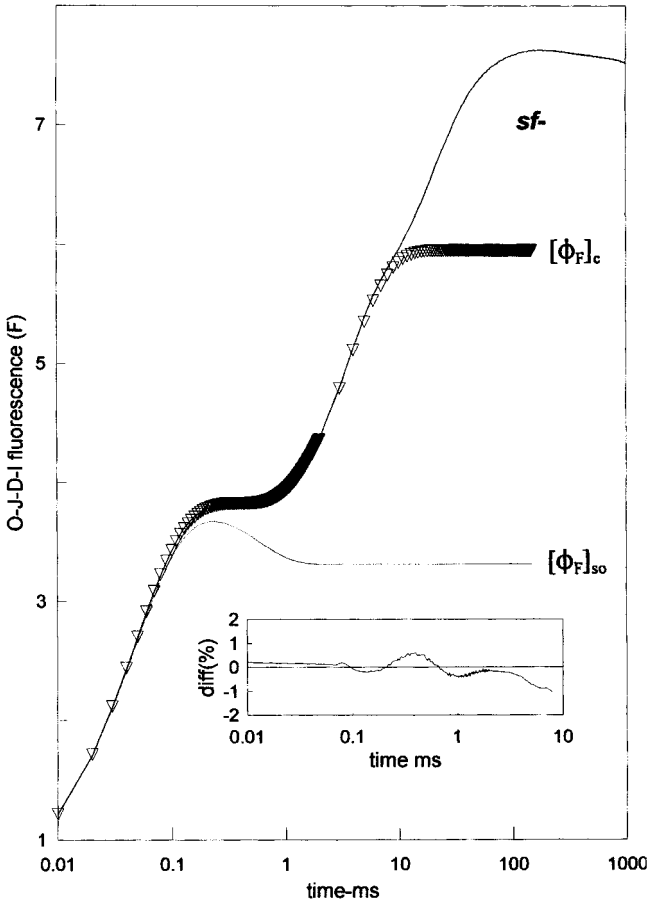

FIGURE 6 Simulated fluorescence induction curves (with symbols) for a dark-adapted Peperomia leaf after a single turnover pre-flash. Simulation was based on the presented model; the time 0 point of the simulated curve was taken as the 10-μs datapoint of the experimental curve (see Fig. 1). The fit parameters are given in Table 1. The lower thin curves in the time region between 0.01 and 5 ms represent the calculated accumulation of semiclosed centers in the absence of their slow conversion in the J-D-I phase. *Insert*: The difference between simulation and experimental datapoints on a percentage scale. Further details are in the text.

Figure 7 and Table 1 show the fit and parameters of the fluorescence induction in the dark-adapted (da-) Peperomia leaf, i.e., in the absence of a single turnover pre-flash, using the same set of equations as in Fig. 6. It is seen that the main differences between the da- and sf-leaf are 1) lower $[\Phi_F]_o$ and $[\Phi_F]_{so}$, 2) an approximately twofold higher rate ($k_{o\text{-}j\text{-}d}$) of the increase in fluorescence in the second and following turnovers, attributed to closure of semiopen centers, and 3) an approximately eightfold lower rate (k_2) of back transfer of these closed centers during the (O-)J-D phase of the fluorescence transient. The difference in these parameter values in the da- and sf-leaf and the relatively invariability of the other fit parameters suggests a major and exclusive effect of a single turnover preillumination on the rate of radical pair recombination in a dark-adapted system. The kinetics of the single turnover induced increase in k_{-1} are unknown. We presume that it is coupled to the charge

TABLE 1 Fit parameters for simulating the fluorescence rise kinetics in a Peperomia and a Chenopodium leaf

	$[\Phi_F]_o$	$[\Phi_F]_{so}$	$[\Phi_F]_c$	O-J $k_{o\text{-}j}$ $(ms)^{-1}$	O-J β	O-J p_{oj}	O-J k_{-q} $(ms)^{-1}$	O-J-D $k_{o\text{-}j\text{-}d}$ $(ms)^{-1}$	O-J-D p_{ojd}	O-J-D k_2 $(ms)^{-1}$	J-I $k_{j\text{-}i}$ $(ms)^{-1}$	J-I p_{ji}	I-P $k_{i\text{-}p}$ $(ms)^{-1}$	I-P p_{ip}
P. metallica														
dark	1.0	2.8 (2.5)	6.2	0.05	0.87	0	0.24	0.5	0	3.8	4.5	0	n.d.	n.d.
+ flash	1.24	3.4 (3.2)	6.0	0.05	0.9	0	0.25	0.9	0	0.3	3.7	0.2	n.d.	n.d.
Chenopodium														
dark	1.0	2.5 (2.3)	5.0	0.15	0.9	0	0.15	0.6	0.4	4	6.0	n.d.	n.d.	n.d.
+ flash	1.13	2.9	4.5	0.14	0.75	0	0.15	1	0.2	3	4	n.d.	n.d.	n.d.

The meaning of the parameters is explained in the text. The numbers in parentheses in the third column from the left refer to the level of $[\Phi_F]_{so}$ associated with the β-value in column 6.

transfer ($S_1 \rightarrow S_2$) in the S-complex at the donor side or between Q_A and Q_B at the acceptor side. These transfers occur with time constants in the range between 10^4 and 10^5 s^{-1}. As said before, the unknown time response of the change in k_{-1} upon excitation of a dark-adapted system makes the data fit with the equations given above less accurate that in an sf-leaf because of this light-dependent change of k_{-1}.

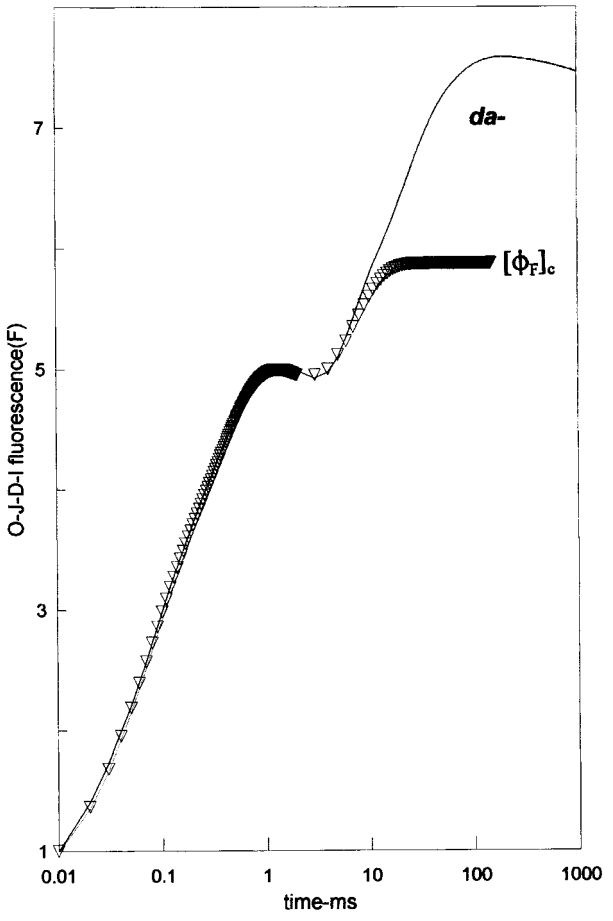

FIGURE 7 Simulated fluorescence induction curves (with symbols) for a dark-adapted Peperomia leaf. The fit parameters are given in Table 1. Further details are in the legend of Fig. 6 and in the text.

A preillumination flash apparently has hardly an effect, if at all, on $k_{o\text{-}j}$, β, or any of the other parameters. It is interesting to note that the rate constant of closure of a semiopen center is about 10 and 5% of that of the (semi) closure of an open center in an sf- and a da-leaf, respectively. The rate of exciton trapping in an RC is in a first approximation equal to the product of the rate constant of its conversion ($k_{o\text{-}j}$ and $k_{o\text{-}j\text{-}d}$, respectively) and the accompanying increment in fluorescence yield, i.e., the variable fluorescence yield $[\Phi_F]_{so}/[\Phi_F]_o - 1$ and $[\Phi_F]_c/[\Phi_F]_{so} - 1$, respectively.

Figure 8 shows the fluorescence induction curves of dark-adapted Chenopodium chloroplasts (thylakoids) in the absence and presence of 30 μM DCMU and the effect thereupon of a single turnover saturating light flash given $\sim$1 s before the start of the fluorescence measurement. The figure is presented in particular to show the effect of the pre-flash on the initial fluorescence level at $t = 10$ μs, which, in the presence of DCMU, has increased to a value close to, if not identical with, $[\Phi_F] = 0.5 * [[\Phi_F]_c - [\Phi_F]_o] = [\Phi_F]_{so}$. It was found (data not shown) that this increase from $[\Phi_F]_o$ to $[\Phi_F]_{so}$ in the presence of DCMU is hardly affected if a few more flashes are given and has a dark recovery with a halftime of 10–15 s. Other details will be dealt with in a separate paper (Rodrigues, to be published).

DISCUSSION

The fluorescence experiments with dark-adapted leaves show an intriguing effect of flash preillumination on the chlorophyll *a* fluorescence yield and the initial phases of its induction kinetics in a high intensity 1-s multiturnover light pulse. Inasmuch as the experiments are similar or comparable, they confirm experiments with intact chloroplasts (Schreiber and Neubauer, 1987) and leaves of other plant varieties (Strasser and Strasser, 1998). Experiments with

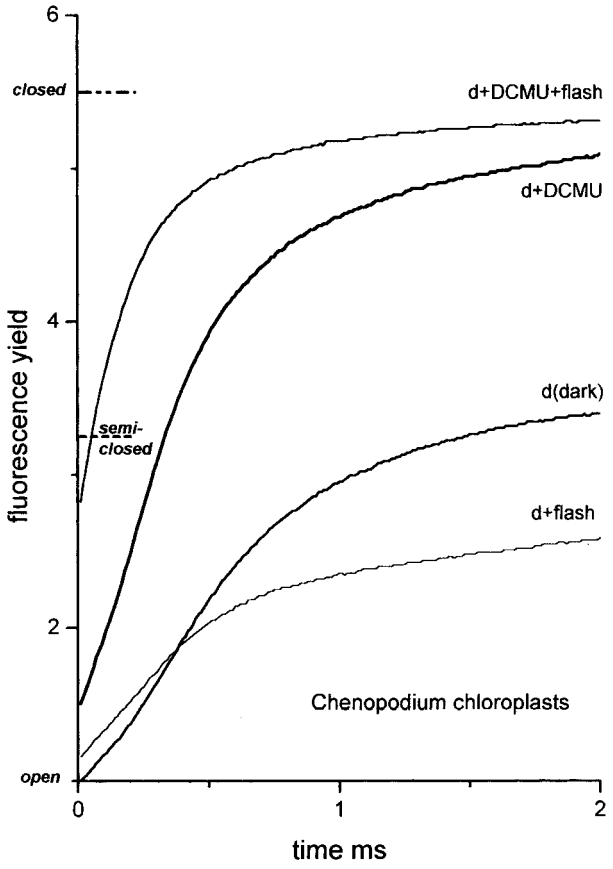

FIGURE 8 Changes in the fluorescence yield in a suspension of dark-adapted broken Chenopodium chloroplasts (20 μg ml^{-1}) upon excitation with a 1-s light pulse of ~600 W m^{-2} intensity in the absence (*lower two curves*) and presence (*upper two curves*) of 30 μM DCMU. In either case, the chloroplasts were preilluminated with a single turnover flash (indicated at the curves with "+flash") or not. In the case of a pre-flash, the onset of the 1-s multiturnover light pulse was given 1.5–2 s after the pre-flash. The maximum fluorescence yield in the presence of DCMU was at 5.5. This level is indicated at the *y* axis with "closed." The "semiclosed" level then is at 3.25, as indicated also. Note that the fluorescence level after a pre-flash in the presence of DCMU is somewhat below the level indicative for semiclosed RCs. (See further the text).

thylakoids (cf. Fig. 8) show strong similarities in effects with those illustrated here for intact leaves (Rodrigues, private communication). The main effects of single-flash preillumination of a dark-adapted system show up as 1) 15–25% increase in the dark fluorescence yield $[\Phi_F]_0$ at the onset of a light pulse (Figs. 2–4 and 8), 2) change in the initial multiphasic and transient O-J-D rise except for a nearly unaltered initial rate (Figs. 2 and 3), and 3) a very low rate (of the order of min^{-1}) of dark reversion of these effects (Fig. 4). In addition, but not dealt with in the present study, the effect mentioned under 1) shows a four-periodic modulation with flash number (Schreiber and Neubauer, 1987; Strasser and Strasser, 1998).

The present so-called three-state model of energy trapping in PSU, which is conceptually different from models used so far, provides a means to interpret and quantify these effects. A schematic visualization with the two-step closure of an initially open RC is given in Fig. 9. It defines and discriminates between open, semiopen (-closed) and closed reaction centers. A semiopen (-closed) state refers to a dynamic state, capable of charge separation, with Q_A reduced and with a low trapping efficiency because the rate of electron transfer at donor and acceptor side (k_s and k_q, respectively) in this state is relatively small as compared to the rate of radical pair recombination (k_{-1}). The concept provides tools for a quantitative description of the fluorescence yield of a system with 100% open and semiopen centers ($[\Phi_F]_o$ and $[\Phi_F]_{so}$, respectively) in relation to the efficiencies of exciton transfer to $[\Phi_p]$ and radical pair recombination $[\Phi_{rp}]$ in the reaction centers.

In a multiturnover light pulse, the fluorescence will rise from the lowest level F_0 (all centers open) to its maximum level F_M upon completion of the trapping (all centers closed). Suppose that, at an intermittent stage of the trapping process, the fractions of open (o), semiclosed (so), and closed (c) are $\alpha:\beta:\gamma$. Then the fluorescence yield at that stage will be given by

$$\Phi_F = \alpha[\Phi_F]_o + \beta[\Phi_F]_{so} + \gamma[\Phi_F]_c, \qquad (5)$$

in which $[\Phi_F]_o$, $[\Phi_F]_{so}$, and $[\Phi_F]_c$ designate the fluorescence yield with 100% RCs in the open, semiopen, and closed state, respectively, and ($\alpha + \beta + \gamma = 1$). Here we see that the commonly used variable $[\Phi_F] - [\Phi_F]_o$ (i.e., the variable fluorescence F_v per unit of I) is dependent on the α/β- and $[\Phi_F]_o/[\Phi_F]_{so}$-ratio, and, thus, cannot easily be related to the ratio between open and closed centra with Q_A oxidized and reduced, respectively. Two of the extreme cases with $\alpha = 1$ (all RCs open) and $\gamma = 1$ (all RCs closed) are assigned as hitherto adopted, with $\Phi_F = [\Phi_F]_o$ and $\Phi_F = [\Phi_F]_c = [\Phi_F]_m$ for $\alpha = 1$ and $\gamma = 1$, respectively. Another special case is considered now when all centers are in the semiopen state ($\beta = 1$) and $\Phi_F = [\Phi_F]_{so}$. The condition for a quasistationary state with nearly 100% semiopen RCs is met when the rate of Q_A^- and P^+-reduction (k_q and k_s, respectively) are small as compared to k_{-1} and, consequently, the probability for radical-pair recombination $\Phi_{rp} = \Phi_{rp}^{so}$ ($= k_{-1}/(k_{-1} + k_q + k_s + k_d)$) ~ 1. This condition is promoted after one turnover in a high-intensity light pulse (Figs. 2 and 3), and is likely to be reached after a single turnover flash in the presence of DCMU at the onset of a light pulse thereafter (Fig. 8). Thus, for the quasistationary state at which, in a strong light pulse (Figs. 2 and 3) or a single (pre-)flash in the presence of DCMU (Fig. 8), all RCs have been transformed into the semiopen state ($\beta = 1$):

$$\Phi_F = [\Phi_F]_{so} = 1 - \Phi_p * (1 - \Phi_{rp}/2) \sim 1 - \Phi_p/2.$$

In the hitherto used concepts, the relatively low fluorescence yield in PSUs with nearly 100% Q_A reduced, which therefore were identified as being closed, was attributed to

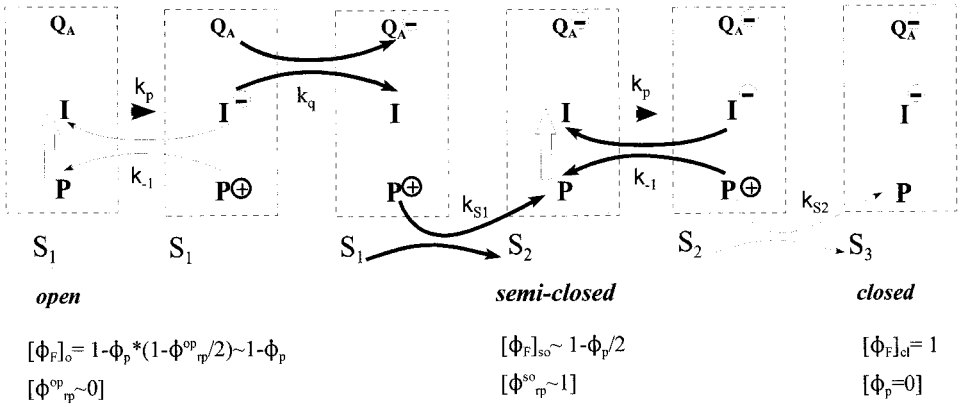

FIGURE 9 The three-state model of energy trapping in PSII under physiological (in vivo) conditions. Schematic visualization of six particular stages of the two-step closure of an open RC (*dashed rectangular*) in a multiturnover light pulse in a dark-adapted system ($S = S_1$). Electron transfer to P^+ at the donor side after a first and second turnover causes transfer from $S_1 \rightarrow S_2 \rightarrow S_3$. Open vertical and closed horizontal straight arrows represent exciton transfer (rate constant k_p) and associated charge separation in the RC. The bent closed arrows mark electron transfer. The rate constants of the pertinent reactions are designated with k_q (Q_A-reduction), k_{-1} (radical pair recombination), and $k_{S1(2)}$ (P^+-reduction by Y_Z with $S = S_1$ and S_2, respectively). Right- and left-oriented arrows symbolize the forward and backward transfer of an RC state, respectively. Φ_F, Φ_p, and Φ_{rp} are efficiencies for fluorescence emission, exciton transfer, and radical pair recombination, respectively. The thickness of an arrow is qualitatively related with the efficiency of the process or reaction it represents. Boxes 1, 4, and 6 (viewed from the left) represent the open, semiopen (-closed), and closed state, respectively. Boxes at position 2, 3, and 5 are transient closed states, which are transferred efficiently in the semiclosed state with transfer times in the submicrosecond time domain. These transient states are beyond the 10-μs time resolution of the fluorescence detection method (PEA, Hansatech) that has been used. The present scheme illustrates the situation at which the excitation rate $(\sim 100\ \mu s)^{-1}$ is high as compared to the rate of Q_A^--oxidation ($k_{-q} \sim (250\ \mu s)^{-1}$). At lower excitation rates, the figure is representative for a system in the presence of DCMU; the lifetime of the semiopen state in the presence of DCMU is about 10 s (data not shown). The figure illustrates that at least two turnovers are required for stationary closure of an RC, the first and second step (semi- and full closure, respectively) of the closing mechanism occur with a large difference in trapping efficiency, and in the presence of DCMU the RCs of the system are transferred by one flash into a quasistationary semiclosed state with the initial fluorescence yield of the system equal to $\Phi_F = [\Phi_F]_{so} = 1 - \Phi_p * (1 - \Phi_{rp}^{so}/2) \sim 1 - \Phi_p/2$. Further explanations are in the text.

either P^+ quenching (Butler, 1972) or to limitation of electron transport at the donor side of PSII (Schreiber and Neubauer, 1987). In the present three-state model, this low fluorescence is attributed to a high population of PSUs with semiclosed centers. With a 100% population density of these PSUs, the fluorescence yield can be approximated to be $[\Phi_F] = [\Phi_F]_{so} = 0.5 * [[\Phi_F]_c - [\Phi_F]_o]$, i.e., 50% of the maximal yield when all centers are closed. In this context, it is of interest to refer to recent work (Kolber et al., 1998), which shows that, using a novel fast repetition rate technique, the increase in fluorescence yield toward a first stationary level was shown to be independent of the number of single turnovers. Varying this number from 1 to 4 did not alter the variable fluorescence yield, which was 65–70% of the maximum yield in these turnovers. This is, indeed, about 50% of the maximal attainable yield and therefore would agree with the present model, which predicts that this intermediate yield originates from semiopen centers. The experiment shown in Fig. 8 nicely illustrates that, with 100% of the RCs in a relatively long-lived semiclosed state (i.e., with Q_A fully reduced) the fluorescence yield is about half the yield of the system with all centers closed.

The present model on a relation between efficiencies for exciton transfer (Φ_p), trapping ($\Phi_{tr} \sim 1 - \Phi_{rp}$), and fluorescence emission (Φ_F) provides reliable parameters for quantifying this relation and to simulate to some extent the kinetics of the fluorescence induction upon a multiturnover high-intensity light pulse in the time range beyond 10 μs. The relation (Eq. 1) emphasizes that stationary closure of an RC requires at least two turnovers (Figs. 4 and 9), and that Φ_F is dependent on Φ_p and Φ_{rp}. The novelty and basic value of the model and of Eq. 1, which is a principle and straightforward modification of the Vredenberg–Duysens equation (Vredenberg and Duysens, 1963), is that it quantifies a relation between Φ_F and Φ_{rp} under conditions at which Φ_p does not alter. Thus, according to Eq. 2 and with a (constant) value of $\Phi_p = 0.83$ (data from Table 1), an ~22% lower value of $[\Phi_F]_0$ in a dark-adapted leaf as compared to a pre-flashed leaf is due to a difference in Φ_{rp} in their open reaction centers of about 0.11. A difference of this size of Φ_{rp}, combined with the observation (Fig. 2 and Table 1, columns 5 and 9) that the initial rate of closure of open ($k_{o\text{-}j}$) and of semiopen centers ($k_{o\text{-}j\text{-}d}$) in a dark-adapted leaf differs by two orders of magnitude is conclusive with an approximately tenfold lower rate of radical-pair recombination in a dark-adapted system, with k_{-1} of the order of $10^7\ s^{-1}$.

The 10–15% decrease in exciton trapping efficiency (Φ_{tr}) in an open RC of a dark-adapted chloroplast after a single turnover pre-flash might be related to what hitherto was attributed to the formation of so-called inactive centers (Lavergne and Leci, 1993). Photocurrent measurements

have indicated that the number of excitable RCs in a train of single-turnover flashes given to a dark-adapted chloroplast is independent of the flash number. Thus, the lower trapping efficiency in RCs after a first flash is likely to be due to an increase in k_{-1} rather than to the formation of inactive centers. The fact that a pre-flash has not caused a change in the rate ($k_{o\text{-}j}$) of the initial O-J rise (Table 1, column 5), notwithstanding an ~10% decrease in the rate of exciton trapping, would suggest an increase in the absorption cross-section caused by the flash preillumination. Such change in cross-section could have occurred in association with the single flash-induced lumen contraction observed in a dark-adapted chloroplast (Vredenberg et al., 1998a,b).

An important fact, which follows from measurements of submillisecond fluorescence kinetics and the model, is that the dark-adapted open state of the RC after it has made one (or more) turnovers has changed its properties with a low rate of reversibility.The present data and analysis suggest evidence that the rate of radical pair recombination after one turnover has increased about one order of magnitude concomitantly with an increase in $[\Phi_F]_0$. The rate of charge recombination of the radical pair has been suggested to be controlled by electric fields of dipoles in the vicinity of the RC (Dau and Sauer, 1991, 1992). A first experimental hint for the likeliness and effectiveness of this kind of interaction has come from measurements of flash-induced photocurrents in combination with measurements of (changes in) $[\Phi_F]_0$ in *Peperomia metallica* (van Voorthuysen et al., 1997; Vredenberg et al., 1998a,b). These measurements showed, for a single chloroplast, a decrease in the electrical conductance of the thylakoid lumen and, for a leaf, a 15–25% increase in $[\Phi_F]_0$, both saturable by a single turnover flash and with a dark recovery in the time domain of minutes. The lumen conductance change has been interpreted to be caused by a contraction of the lumen, which will alter the proximity of membrane proteins and is likely to be accompanied by changes in energy transfer within and between LHCs. The change in k_{-1} is interpreted to be due to an altered electrostatic interaction of dipoles in the vicinity of the RC (Vredenberg et al., 1998a,b). Therefore, the dark-adapted open state can only be taken as a reference for the semiopen and closed states if the change in k_{-1} is considered.

The ratio between variable ($F_v = F - F_o$) and maximal fluorescence (F_m) in general is taken as the measure for the yield of photochemistry of PSII ($\Phi p = F_v/F_m$). This now appears to be in disagreement with the three-state trapping model of PSII. Moreover, F_o, usually taken as the minimal fluorescence in a dark-adapted system, is strongly light dependent with a very low light requirement and a low rate of reversibility. Thus, it cannot be excluded, and preliminary experiments have shown (paper in preparation), that the maximal fluorescence F_m at the P-level, measured with a saturating light pulse of a few hundreds of milliseconds duration is from an energetic and/or conformational state of the antenna–RC system that is different from the original dark-adapted state. The difference among others shows up as substantially higher F_o due to a light-dependent increase in k_{-1}. To circumvent interference of these changes with the measurement of fluorescence parameters, the fluorescence induction in a dark-adapted state should be monitored shortly after a few single turnover flashes (even one flash would be sufficient) have been given. These pre-flashes will cause a subtle but saturated change in the energetic state of the system, reflected by a decrease in the lumen conductance and an increase in k_{-1}, which is reflected by changes in the O-, J-, and D- levels. In that case, the open state of the reaction center is characterized by an electrostatically stabilized value of k_{-1}. The fluorescence at the J-level (F_J), which reflects accumulation of a large fraction of PSUs with semiopen RC, then becomes closest to the fluorescence yield $[\Phi_F]_{so}$ ($= 0.5 * [[\Phi_F]_c - [\Phi_F]_o]$) of this population and is likely to be less hampered by the input dose. However, no data are available as yet whether such an effect can be excluded. They appear to exist during J-I-P phase, which, for instance, can be concluded from other published work (Strasser et al. 1995). The fluorescence at the I-level (F_I) is interpreted to be of the system with 100% of the RCs closed, i.e., with $[\Phi_F]_1 = F_I/I = [\Phi_F]_c = 1$. The I-P rise, which is found to be inhibited by DCMU (Rodrigues, private communication), needs further evaluation. The change in fluorescence during this phase has been suggested to be associated with changes in quenching by the plastoquinone pool (Vernotte et al., 1979).

In conclusion, our data show the necessity and usefulness of a three-state energy trapping model of PSII that takes into account an electrostatic effect of local charges in the vicinity of the RC affecting the rate of radical pair recombination with a subtle light regulation. The model provides tools for quantifying and simulating the multiphasic fluorescence induction in photosynthetically competent tissues and preparations. It is recommended that the measuring protocols in the widely used PAM- and PEA-fluorescence instruments are accommodated with a routine to ensure that F_0 and F_m (PAM) or O-J-I-P (PEA) measurements are also done with reference to a dark-adapted sample to which one saturating flash has been given.

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