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Bioelectrochemistry 68 (2006) 81 – 88

Bioelectrochemistry

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On the sub-maximal yield and photo-electric stimulation of chlorophyll *a* fluorescence in single turnover excitations in plant cells

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Received 21 January 2005; received in revised form 3 May 2005; accepted 9 May 2005
Available online 20 July 2005

Abstract

A set of expressions is derived which quantifies the chlorophyll fluorescence yield in terms of rate constants of primary light reactions of PSII, the fraction of open and semi-open RCs and of the electric field sensed by the RC in the thylakoid membrane. The decay kinetics of the chlorophyll fluorescence yield after a single turnover excitation in the presence of DCMU show at least two components, one reversible within approx. 1 s and one with a dark reversion lasting more than 30 s. The latter is attributed to photochemical quenching; the fast component is interpreted to be associated at least partially with photo-electrochemical control. It will be illustrated that (i) the sub-maximal fluorescence yield in single turnover excitation is associated with semi-closure of RCs, (ii) the trapping efficiency of semi-closed centers is less than 50% of that of open centers and (iii) the fluorescence yield of antennas with semi-closed RCs has the highest sensitivity to changes in strength of photo-electric fields.

Keywords: Chlorophyll fluorescence yield; Single turnover excitation; Photochemical quenching; Electric field effect; Double hit trapping mechanism

1. Introduction

Measurement and quantitative analyses of variable chlorophyll *a* fluorescence in green plant cells and chloroplasts are a promising non-invasive means for evaluating various photosynthetic parameters, in particular those associated with photosystem II (PSII) [1–9]. Amongst those are rate constants of energy transfers and-dissipations in antennas and reaction center (RC) as well as of intersystem connectivity within PSII units and of electron transfers at donor and acceptor side of PSII [6,7]. Fluorescence emission is competitive with other dissipative pathways (collectively termed non-photochemical quenching) and with the energy that is photochemically trapped in the RCs of PSII (photochemical quenching).

The kinetic pattern of the fluorescence emission signal (F) starting from an initial level Fo (maximal photochemistry, high photochemical quenching) and rising to the maximal level Fm (saturated photochemistry, low photochemical quenching) upon excitation with a multi-turnover light pulse shows a well-documented transient denoted as

Abbreviations: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; Fm, fluorescence level of system with 100% closed PSUs in dark-adapted state; Fo, fluorescence level of system with 100% open PSUs in dark-adapted state; k_{-1}, rate constant of radical pair recombination; k_{AB}, rate constant of Q_A-oxidation; k_d, rate constant of non-radiative radical pair transfer; k_e, rate constant of Q_A photoreduction (charge stabilization at acceptor side); k_f, rate constant of fluorescence emission; k_L, excitation rate of photosystem in light pulse; k_t, rate constant of photochemical trapping (charge separation) in PSII; k_w, rate constant of non-photochemical energy losses; $k_{yi,\ si}$, rate constant of P^+- and Y_Z^+-reduction, respectively for OEC in $S=S_i$-state ($i=1,..4$); N, antenna size of PSII; OEC, oxygen evolving complex; ODE, ordinary linear differential equation; P680 (or P), primary electron donor of PSII; Phe(or Ph), pheophytin, primary electron acceptor of PSII; Ψ, electric potential difference (dimensionless, 1 unit corresponding with electrochemical value $RT/F \sim 25$ mV); PSII(I), photosystem II(I); PSU, photosynthetic unit (refers mostly to PSII); Q_A, primary quinone acceptor of PSII; Q_B, secondary quinone acceptor of PSII; RC, reaction center of PSII; TSTM, three-state trapping model; VMC, valinomycin; Y_Z, secondary electron donor of PSII.

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doi:10.1016/j.bioelechem.2005.05.001

O–I_1–I_2–M [1] or O–J–I–P [2]. The subsequent transients in high intensity light pulses are at about 1, 100 and 500 ms. For a large variety of leaves and isolated chloroplasts, the relative fluorescence F/Fo at O-, J-, I- and P (M) is found at 1 and around 3, 5 and 6, respectively [2,5]. An alternative and commonly used parameter for characterizing the photosynthetic competence of a leaf or chloroplast preparation is the variable fluorescence Fv ($=F-Fo$) relative to the maximal fluorescence Fm at M (or P): Fv/Fm. According to this definition $Fm/Fo \sim 6$ corresponds with $Fv/Fm \sim 0.83$. In general, Fv/Fm-values around 0.8 ($Fm/Fo \sim 5$) are considered to be representative for high performance and competence of the photosynthetic machinery in leaves, green cells and isolated chloroplasts.

The bi-phasic kinetic profile of the release of photochemical quenching with about equal increments from $F/Fo=1$ to about 3 and subsequently to about 5 has been ascribed to a photochemical and a thermal phase of the fluorescence induction curve $F(t)/Fo$, respectively [10,1]. The photochemical quenching is attributed to oxidized forms of the primary quinone acceptor Q_A and of the primary donor P680 of PSII. Release of quenching occurs in association with light-driven Q_A- and P^+-reduction (i.e. Q_A- and Y_z^+-formation) at the acceptor and donor side of PSII, respectively. It has been proposed, based on similarity between (S state-dependent) rate constants of quenching release at high excitation rate and of Y_z^+-reduction, that Y_z^+ (the oxidized form of the secondary donor tyrosine of PSII) is a quencher as well [11]. Until now, there is continuing discussion about the processes that are responsible for the slow fluorescence rise during the thermal phase. Current visions consider that it is associated with release of quenching by quenchers amongst which plastoquinone [12,13] or other quinones, like Q_B^- [5,14,15] and other as yet unidentified components [16], or alternatively two forms of fluorescence (prompt and recombination) with different properties [17] have been proposed as prime candidates. The thermal (J–I) rise is several fold enhanced in the presence of DCMU causing that it coincides with the O–J phase, giving rise to the rapid rise to the I-level with $F/Fo \sim 5$. An aberrant interpretation of this amply documented phenomenon has been proposed [18].

Recently, an alternative trapping model has been presented [6,11]. It emphasizes that a single hit reduction of Q_A, in contrast to what implicitly and commonly is assumed, does not necessarily result in full closure of the RC. A full closure would imply that charge stabilization or electron trapping efficiency in subsequent excitation (hits) is zero. Briefly stated, the alternative, such called Three-State Trapping Model of PSII (TSTM), considers that RCs with Q_A^-, i.e. with zero charge stabilization at the acceptor side of PSII, are competent of charge stabilization (electron trapping) at the donor side. RCs with one electron stored at Q_A^- and competent of charge separation and stabilization at their donor side have been called semi-open (-closed) centers. In other words, TSTM is based on the hypothesis that the RC of PSII acts as a 2-electron trap, and that full closure of the RC requires at least a double hit. It has been proposed [19] that stabilization of the electron on Pheo-in semi-closed centers is accomplished by sharing the electron with Q_A^- causing the double reduction of Q_A. The possibility of a second electron transfer step in the trapping process of PS2 has been suggested before [20].

The fluorescence time pattern (induction curve) can be quantified with TSTM in terms of rate constants of electron transport at the donor and acceptor side of PSII [11,19]. The O–J part (photochemical phase) is for its major part a reflection of the transition of open into semi-closed (-open) centers. The subsequent slow thermal phase (J–I) is associated with the transition of semi-open into closed centers. The attenuated rate of this transition is due to the low electron trapping efficiency in semi-open centers [21].

It has been reported [15,17] that the fluorescence yield in a single turnover excitation, at least under certain conditions, can be close to the maximum yield reached in multi-turnover flashes. This would be at seeming variance with TSTM, which predicts that a single hit causes semi-closure of an RC associated with half maximal rise in fluorescence yield. Here we present quantitative expressions for of a double hit trapping concept, in which the effect of electrical fields on the fluorescence yield is incorporated in the TSTM. These offer an alternative means for interpreting the sub-maximal fluorescence yield in single turnovers.

2. Material and methods

Experiments were performed on intact leaves of *Chenopodium album* L and chloroplasts isolated thereof of as well as of pea (cv. Premium and Alfa), or barley. Growth and isolation conditions and procedures are identical as published elsewhere [22].

Induction curves of chlorophyll fluorescence were measured with a Plant Efficiency Analyzer (either PEA-, or Handy PEA fluorometer, Hansatech Instruments Ltd, King's Lynn, Norfolk, UK) and viewed with dedicated software. Leaf segments were cut from leaves and fixed in clip holders with circular openings of uniform dimension. Measurements were performed at room temperature. After a 10 min dark adaptation, fluorescence was excited with 1-s pulses of red light (650 nm) emitted with light-emitting diodes at maximal irradiance of about 650 W m^{-2} (approximately 3300 μmol m^{-2} s^{-1}). Thylakoids were suspended in 0.5 ml reaction medium of 0.3 M sorbitol, 50 mM tricine–NaOH (pH 7.6) and 5 mM $MgCl_2$ at a Chl concentration of 20 μg ml^{-1} and kept in the dark for several tens of minutes in the measuring cuvette. DCMU was added in complete darkness at a final concentration of 10 μM. The sample was illuminated with one saturating single turnover flash (Xe lamp, half width <6 μs, Walz, Effeltrich, Germany) and transferred within 1 to 2 s to the sample compartment in the fluorometer. Under the conditions used,

the flashes were approx. 75% saturating (data not shown). Fluorescence data were recorded at a sampling rate of 10 μs in the lower time range between 0.01 and 0.2 ms, and at lower rates in higher time domains. The experimental traces in general represent the averages of three samples each illuminated a single time.

The multi-phasic fluorescence induction curve has been evidenced to be composed of a photochemical and a photoelectrochemical component [19,23]. The photoelectrochemical component, that is seen in the 20–200 ms time range has been found to be inhibited by high concentration of PSII inhibitors, far above the pI_{50} for PSII inhibition [22].

The photochemical phase of the fluorescence curve is simulated using a TSTM-based analysis which describes the fluorescence quenching in relation to energy trapping in terms of rate constants of primary and associated reactions at the donor and acceptor side of PSII [11]. The model enables the trapping process, i.e. the closure of the reaction centers with its successive reaction steps and intermediates to be written in a (multi-) set of ordinary differential equations (ODEs) with rate constants of distinct partial reactions as determinant parameters. Computer-assisted solution of these ODEs in combination with application of proper optimization routines (Mathcad 2001i and elder versions, MathSoft, Inc. Cambridge, Mass., USA) gives the simulation of the experimental curve in terms of reaction rate constants and light excitation rate.

The basic part of the trapping model is illustrated. It is presented in an alternative form, incorporating the photoelectric effects [23–25], and focusing on the sub-maximal fluorescence yield after single turnover excitation [16,26].

3. Results and interpretation

Fig. 1 shows the interaction scheme of the processes that govern energy transfer and dissipation in the antennas and photochemical trapping in the reaction center of PSII. Energy dissipation of excited antenna chlorophylls (Chl*) occurs via fluorescence (k_f) and via non-radiative routes (k_w). The excited reaction center chlorophyll of PSII, P680*, is in excitonic equilibrium with excited states of the N antennas. Energy of P680* is transferred (k_t) and via charge separation photochemically converted into the electrochemical energy of the reaction center radical pair P^+Phe^-. Energy transfer from the radical pair occurs via (i) charge stabilization (electron trapping) at the acceptor and donor side of PSII (rate constants k_e and k_y, respectively), (ii) radical pair recombination (k_{-1}) or (iii) back transfer to the ground state (k_d). The latter might include a route via a triplet state. The particular property of the RC trap of PSII with respect to the relatively low energy difference between P680* and the radical pair, causes that the energy equilibrium between these states is strongly dependent on the strength of the local electric field in the RC. This shows

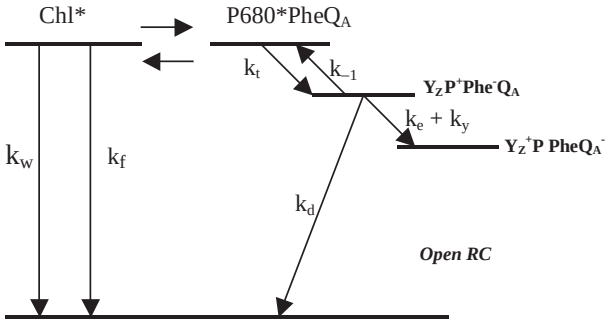

Fig. 1. Scheme of energy trapping in PSII with *open reaction centers*. Rate constants of the reaction steps involved are indicated (see further text). k_w: rate constant of non-photochemical energy losses in antennas, except fluorescence; k_f: rate constant of fluorescence emission in antennas; k_t: rate constant of photochemical trapping (charge separation) in PSII; k_{-1}: rate constant of radical pair recombination in RC; k_e: rate constant of Q_A photoreduction (charge stabilization at acceptor side); k_y: rate constant of P^+-reduction (charge stabilization at donor side); k_d: rate constant of non-radiative radical pair transfer to ground state in RC. The transition of $Y_zP^+Phe^-Q_A$ to $Y_z^+PPheQ_A^-$ with a rate constant k_e+k_y is a formal representation of the sequential transition from $Y_zP^+Phe^-Q_A$ to $Y_zP^+Phe\,Q_A^-$ (with rate constant k_e) and from $Y_zP^+Phe\,Q_A^-$ to $Y_z^+PPhe\,Q_A^-$ (with rate constant k_y) with $k_e>k_y$.

up in the dependence of the ratio k_{-1}/k_t on the electric field (potential). It has been derived [25] that

$$\frac{k_t}{k_{-1}} = e^{(\psi_0-\psi)} \tag{1}$$

in which ψ_0 and ψ are dimensionless and refer to the redox potential difference between P680* and the radical pair and to the electric potential difference, respectively (1 unit corresponding with the electrochemical entity RT/F ~ 25 mV). In the absence of an electric field ($\psi=0$) and with $\psi_0 \sim 4$ (~ 100 mV), $k_t/k_{-1} \sim 55$.

Eq. (2) gives the chlorophyll fluorescence yield in relation to the rate constants of the energy transfer, dissipation and photochemical trapping in antennas and RC (see Fig. 1) and as a function of the potential ψ and fractions θ ($0 \le \theta \le 1$) of RCs with unaffected (θ_1), acceptor side inhibited (θ_2) and both sides blocked ($1-\theta_1-\theta_2$) charge stabilization in the RC of PSII, respectively.

$$\phi_f(\theta_1,\theta_2,\psi) = \frac{1}{1+\frac{k_w}{k_f}+\frac{\left[\theta_1(k_e+k_y)+\theta_2 k_y+k_d\right]}{k_f N}e^{(\psi_0-\psi)}} \tag{2}$$

This equation is modified in comparison with earlier published forms [25,23]. It accommodates, in contrast to these, the effect of non-zero charge stabilization at the donor side, i.e. $\theta_2>0$, when, at full reduction of Q_A, charge stabilization at the acceptor is blocked ($\theta_1=0$). The θ_2-fraction has been called semi-open (-closed) [6]. The scheme of energy trapping in semi-open centers is visualized in Fig. 2.

Time functions $\theta(t)$ of the respective fractions and of the electric trans-thylakoid potential $\psi(t)$ associated with photochemical energy trapping and storage, respectively, deter-

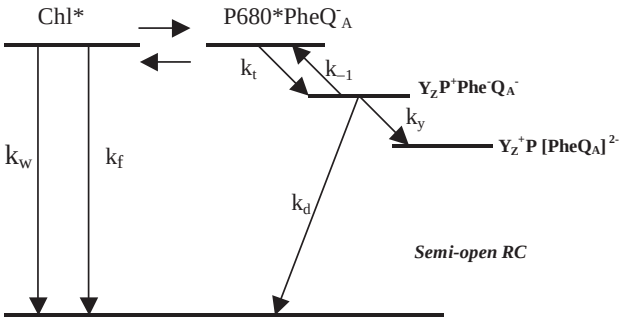

Fig. 2. Scheme of energy trapping in PSII with *semi-open* reaction centers (Q_A reduced). The location of the 2nd electron at the acceptor side is not specified and designated with $[PheQ_A]^{2-}$. See further legend of Fig. 1.

mine the time function of the fluorescence yield $\phi_f(t, \theta_1, \theta_2, \psi)$. According to Eq. (2), the fluorescence yield upon excitation, assuming no change in potential ψ (=0 for a dark adapted state), will rise from a low value at $\theta_1=1$ (open centers) to high(er) value at $\theta_1=\theta_2=0$ (closed centers). We define

$$\phi_f^{open} = \phi_f(1,0,\psi) = \frac{1}{1+\frac{k_w}{k_f}+\frac{(k_e+k_y+k_d)}{k_f N}e^{(\psi_0-\psi)}} \quad (3)$$

$$\phi_f^{semi-open} = \phi_f(0,1,\psi) = \frac{1}{1+\frac{k_w}{k_f}+\frac{(k_y+k_d)}{k_f N}e^{(\psi_0-\psi)}} \quad (4)$$

$$\phi_f^{closed} = \phi_f(0,0,\psi) = \frac{1}{1+\frac{k_w}{k_f}+\frac{k_d}{k_f N}e^{(\psi_0-\psi)}} \quad (5)$$

and, at high potential ($\psi \gg 0$),

$$\phi_f^{max} = \frac{1}{1+\frac{k_w}{k_f}} \quad (6)$$

A comparison of Eqs. (5) and (6) shows that, after saturation of energy trapping when RCs are closed ($\theta_1=\theta_2=0$), the fluorescence yield can further increase from ϕ_f^{closed} to ϕ_f^{max} in response to an increase in the potential ψ. For instance, as will be illustrated below, a potential increase $\Delta\psi=3$ (~75 mV) will bring the fluorescence yield close to ϕ_f^{max}.

We will present and express experimental fluorescence signals in response to a light pulse, $F(t)$, relative to the signal Fo (=$F(0)$) which is measured in a dark-adapted sample ($\psi=0$) approx. 10 μs after the onset of the excitation. The maximal fluorescence is designated with Fm. The following equalities then will hold

$$\frac{F(t)}{Fo} = \frac{\phi_f(t,\theta_1,\theta_2,\psi)}{\phi_f^{open}} \quad (7)$$

and

$$\frac{Fm}{Fo} = \frac{\phi_f^{max}}{\phi_f^{open}}. \quad (8)$$

There is general consensus, in agreement with estimates on ϕ_f^{max}, that the rate constant of fluorescence emission $k_f \sim 0.03$ $(ns)^{-1}$ is about 10% of that of non-radiative dissipations k_w[3]. Equation (6) then gives $\phi_f^{max}=0.091$, i.e. a maximal fluorescence yield of ~9%. With commonly estimated Fm/Fo values of 5 to 6 (see also below), Eq. (8) then would give a minimal dark fluorescence yield ϕ_f^{open} of 1.5 to 1.8%. Fig. 3 gives the fluorescence time curve $F(t)/Fo$ on a logarithmic time scale in a 1 s light pulse of an intact dark-adapted leaf. It shows the well-known kinetic O–J–I–P profile. F/Fo values at the distinct fluorescence levels at 0.01 (O), ~2 (J), ~30 (I) and ~200 ms (P) are 1.0, 3.5, 5.5 and 6.0, respectively. These are average values commonly found in intact healthy leaves of various plants [5]. With Fm/Fo=6 and $\phi_f^{max}=0.091$ one gets (see above, Eq. (8)) $\phi_f^{open}=0.015$. Experimental evidence has been presented [19,25] in support of the hypothesis that the I–P-fluorescence rise in the 30 to 200 ms time domain is a response to the photoelectric trans-thylakoid potential generated by photosystem I (PSI). This would imply that at the I-level, and with $\psi=0$, $F(t)/Fo$ (see figure) corresponds with $\frac{\phi_f^{closed}}{\phi_f^{open}} \sim 5.5$. Evidence is accumulating that a single turnover excitation, which leads to saturation of charge stabilization at the acceptor side (full reduction of Q_A) and, in our concept, to semi-closure of RCs, causes a fluorescence increase which is 40 to 60% of the maximal fluorescence increase in a multi-turnover excitation [16,26,27]. This would mean for the present experiment, when, as an average, the fluorescence increase in a single

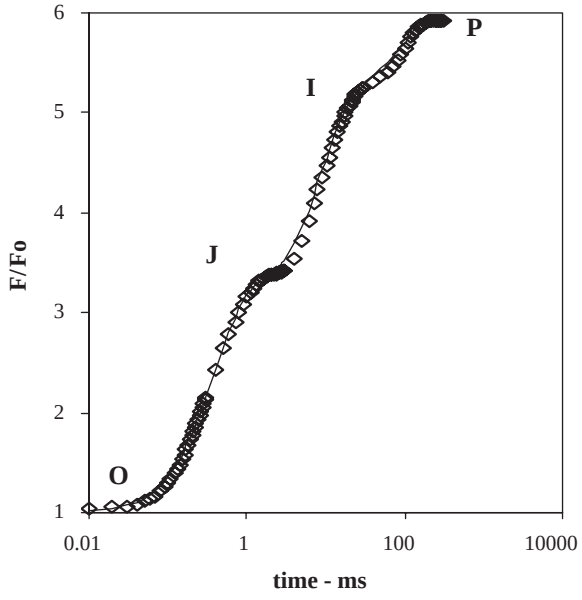

Fig. 3. $F(t)/Fo$ fluorescence kinetics (symbols) of intact pea leaf in 1 s multiturnover light pulse. Solid line is rough simulation with double hit three state trapping model [11].

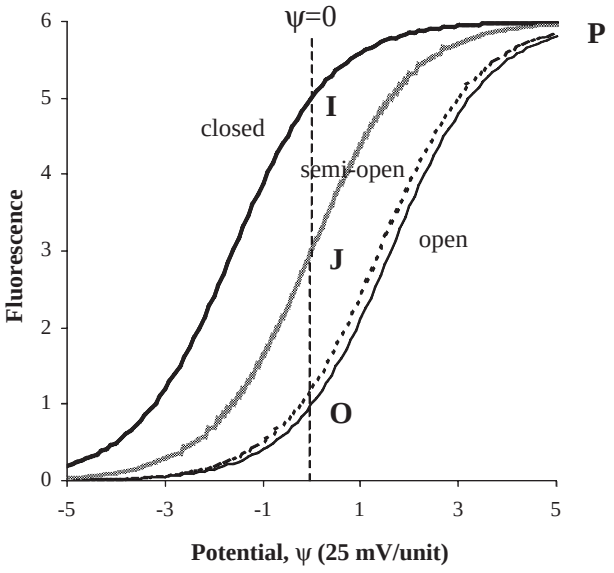

Fig. 4. Dependence of the fluorescence yield on the electric field (potential) ψ for RCs in which charge stabilization at donor and acceptor side of PSII is unimpaired ($\phi_f(1,0,\psi)$, open centers), charge stabilization at the acceptor side is blocked ($\phi_f(0,0,\psi)$, semi-open) and when the stabilization at both sides is blocked ($\phi_f(0,0,\psi)$, closed centers). *X*-axis: 1 unit corresponds with potential $\psi \sim 25$ mV; *Y*-axis: fluorescence signal F/Fo with $F=\mathrm{Fo}=\phi_f(1,0,0)=1$ at $\psi=0$ (see further text).

turnover is assumed to be 50% of the maximal yield, that $\frac{\phi_f^{\text{semi-open}}}{\phi_f^{\text{open}}} \sim 3.5$. This value corresponds reasonably well with the F/Fo value at the J-level (see Fig. 3), and is in agreement with an earlier conclusion that the J-level for the major part is associated with accumulation of semi-open centers [11].

The experimental F/Fo curve and the presumed yield after a single turnover excitation thus give the values of fluorescence yields of the respective RC states and as such the constraints for the actual values of the rate constants of reactions involved in photochemical trapping in the RC of PSII, as depicted in Fig. 1. For example with an antenna size $N=100$ and a rate constant of Q_A-reduction $k_e=3$ $(\mathrm{ns})^{-1}$, application of Eqs. (3)–(6) gives a unique solution for the rate constants (in ns^{-1}) of k_y and k_d (see Fig. 2) and for ψ_0 (mV) as shown here for two extreme, but commonly found, F/Fo values at the J- and I-level in intact leaves.

N	ϕ^{so}/ϕ^0 [at J]	ϕ^{closed}/ϕ^0[at I]	k_f	k_w	k_e	k_y	k_d	ψ_0 (mV)
100	3.5	5.5	.03	0.3	3	0.44	0.06	96.2
100	3.0	5.0	.03	0.3	3	0.60	0.15	96.2

Fig. 4 gives the plots of the fluorescence yield $\phi_f(\theta_1, \theta_2, \psi)$, normalized relative to Fo ($=\theta_f(1,0,0)$)=1, against the electric potential ψ in the range from −5 to 5 (−125 to 125 mV) for open ($\theta_1=1$), semi-open ($\theta_2=1$) and fully closed ($\theta_1=\theta_2=0$) RCs using Eqs. (3)–(6) and the rate constants dictated by the experimental $F(t)/\mathrm{Fo}$ curve (Fig. 3). It shows the strong dependence of the PSII chlorophyll fluorescence yield on the electric field in the vicinity of and sensed by the RC. This potential may originate from local charges (single or dipoles) or from the trans-thylakoid electric potential. It illustrates that even a small increase in ψ, or decrease in ψ_0 will cause a change in the fluorescence yield. For instance the 15–25% increase in Fo in a dark-adapted sample upon a single turnover excitation that has been shown to persist during minutes has been attributed to a change in the static electric field sensed by the RC [6]. This effect has been simulated in the figure for $\Delta\psi=0.4$ (~ 10 mV) and is associated with $\sim 22\%$ increase in Fo. The upper curve illustrates the effect of an increase in the potential on the fluorescence yield in closed centers when, at the I-level, the photochemical quenching has been released ($\phi_f^{closed}=\phi_f(0,0,0)=5$, Eq. (5)). An increase in the potential $\Delta\psi=3$ (~ 75 mV) will then increase the fluorescence yield towards $F/\mathrm{Fo}=\phi_f$ (0,0,3) ~ 6.5 at the P-level. Particular attention deserves the strong and nearly linear effect of the potential on the fluorescence yield in semi-open (-closed) RCs. The figure shows that at the J-level ($\phi_f(0,1,0)=3$, Eq. (4)) a potential change $\Delta\psi=3$ (~ 75 mV) will cause an increase in the yield to a value far above 5. This effect should be kept in mind when changes in the fluorescence yield in single turnover excitations are studied. As single

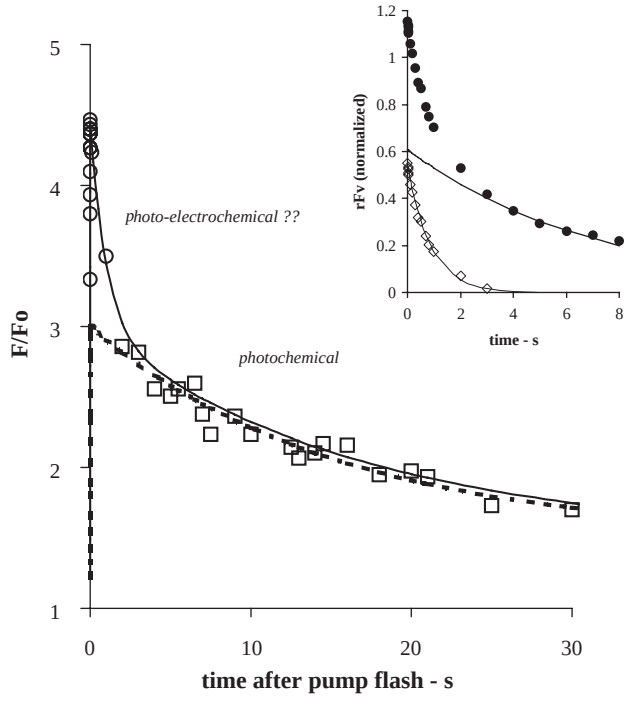

Fig. 5. F/Fo fluorescence signal (boxes) probed in the time interval between 2 and 30 s after a single turnover excitation of *Chenopodium* chloroplasts in the presence of DCMU. F and Fo are the initial fluorescence signals measured at approx. 10 μs after the onset of 1 s a light pulse; Fo is the signal in a dark adapted control sample (absence of DCMU). The dashed curve is the nearest single-exponential fit of the data points. Open circles are from similar experiments in the 0.02 to 1000 ms time domain reproduced from Schreiber [15,17]. The solid line is the exponential fit of these data points and superimposed on the dashed curve. Insert shows similar data published by Roberts et al. [31].

turnovers have been shown to generate transient trans-thylakoid photopotentials several tens of mV in magnitude [28–30], one might expect a substantial contribution of these photoelectric events on magnitude and kinetics of the fluorescence kinetics. So far, the effect of photoelectric potentials on fluorescence kinetics upon single turnover flash excitation has been ignored [15–17].

Fig. 5 shows the decay of the initial fluorescence F in a short pulse, expressed as F/Fo, probed in the time interval between 2 and 30 s after a single turnover excitation of chloroplasts in the presence of DCMU. F is, like Fo for the control (absence DCMU) in the dark, the fluorescence signal at approx. 10 μs after the onset of the excitation pulse. The decay of the probed fluorescence is exponential, completed after more than 25 s and, when extrapolated to $t=10$ μs after the flash, originates from an initial amplitude $F/\mathrm{Fo}\sim 3$ [6]. These pump-probe experiments were hampered by a requirement of manual handling of samples after the flash limiting the shortest time interval between pump and probe to ~2 s. However, when supplemented with data of similar pump-probe experiments in the 0.020 to 1000 ms time domain reported by Schreiber [15], an additional fast component is obvious. The kinetic analysis indicates the following. (i) The F/Fo signal probed after a single turnover pump flash in the presence of DCMU has a maximum with $F/\mathrm{Fo}\sim 4.6$ probed at ~0.1 ms after the pump flash. (ii) The decay curve cannot be fitted with a single exponential function; a fast exponential decay with rate constant $\sim 1\ s^{-1}$ is followed by a second component with $F/\mathrm{Fo}\sim 3$ and rate constant $\sim 0.1\ s^{-1}$. (iii) The reproduced curve closely resembles one representing the replotted results (see insert in figure) of similar pulse-probe experiments in the presence of 1 μM DCMU in the time domain $10^{-4}-10$ s (see [31], Fig. 1A).

4. Discussion

The notion that charge stabilization at the donor side of PSII is likely to occur when electron trapping (stabilization) at the acceptor side has become zero upon Q_A-reduction, offers an alternative and simple explanation for the sub-maximal chlorophyll fluorescence yield induced by a single turnover excitation in leaf cells and chloroplasts. Several reports have dealt with this surprising phenomenon [6,16,26]. Various explanations and interpretations have been postulated, based on the implicit assumption that charge stabilization in RCs with reduced Q_A is zero. The average 40–60% magnitude of the yield in a single saturating flash relative to a maximal yield in multiturnover excitations has been ascribed to photochemical quenching of quenchers amongst which quinones and other, as yet unidentified, components have been proposed as major candidates (for Refs., see [16]). In addition, the lower yield in single turnovers is thought to arise from non-photochemical quenching in either RC [15] or antennas [16]. A recently proposed so-called three state trapping model (TSTM) of PSII incorporates a non-zero charge stabilization in RCs in which e-transport at the acceptor side was blocked due to the presence of Q_A[6,11]. Like TSTM, other models have been proposed to provide a quantitative description of the fluorescence kinetics in light pulses [4,7,8,16,17,32]. In all of these, it is implicitly assumed that (i) the electron trapping efficiency in reaction centers with reduced Q_A is zero which is synonymous with considering them as being closed, and, except in [32], (ii) the fluorescence changes are independent on changes in intra or trans membrane electric fields.

As illustrated in Figs. 1 and 2, and in agreement with data obtained from experimental time curves of the variable fluorescence yield in an illuminated intact leaf (Fig. 3), a reduced fluorescence yield in a single turnover excitation is predicted (Eq. (4)) when charge stabilization in semi-open centers associated with primary electron transfer at the donor side of PSII is considered. An approx. 50% yield is calculated when P^+ reduction (Y_Z oxidation) occurs with rate constant $k_y=0.6\ (\mathrm{ns})^{-1}$. This value is in the range reported for the rate constant of P^+-reduction under condition at which the oxygen evolving complex is in the S_1 or S_2 state [33,34]. The conclusion that the fluorescence yield after a single turnover is half maximal is not in conflict with the results of the pump-probe experiments illustrated in Fig. 5, if it is assumed that the slow phase of the bi-phasic fluorescence decay after single turnover excitation in the presence of DCMU is associated with photochemical quenching. The alternative explanation that this phase reflects only a 50% fraction of photochemically active RCs is unlikely. The other fast decaying fraction cannot easily be identified as one that is photochemically involved in a back reaction with an S-state of the OEC. We propose the

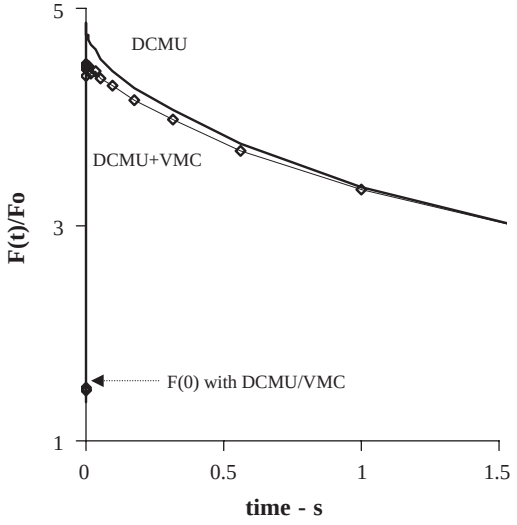

Fig. 6. F/Fo fluorescence signal probed in the time interval between 100 μs and 1.5 s after a single turnover 30 μs excitation of *Pea* chloroplasts in the presence of DCMU (upper curve) and VMC+DCMU (lower curve), respectively. Fo is the signal, measured at 100 μs after the onset of excitation flash in a dark-adapted control sample (not shown). Note also the effect of DCMU on the initial fluorescence level $F(0)$ with, in this case $F(0)/\mathrm{Fo}\sim 1.5$ (without effect of VMC). [reproduced from Fig. 2 in Ref. 35].

hypothesis that at least part of the fast fluorescence component after a single turnover excitation presumably is associated with a photoelectrochemical response of the fluorescence to photoelectric events that occur in the membrane and thylakoid lumen. As Fig. 4 predicts, a transient trans-thylakoid potential of several tens of mV will give rise to a substantial rise in the fluorescence yield in (semi-open) centers with fully reduced Q_A. Patch-clamp studies with intact chloroplasts of Peperomia metallica have provided experimental data that show single turnover-induced photopotentials of this size and with a time pattern similar to of the fluorescence decay of the fast component (i.e. in the 0.1 to 1 s time range [28,35]). The hypothesis has received support from preliminary results of pump-probe experiments in which the effect of membrane-permeabilizing agents on the fluorescence kinetics after single turnover excitation was studied in the presence of DCMU [36]. These experiments have shown, as illustrated in Fig. 6, the inhibition of a fast fluorescence component in the 0.1 ms to 1 s time range by valinomycin and gramicidin, whereas the 2 major components in the 0.1 to 10 s range, likely to be associated with regeneration of photochemically converted Q_A, were not affected. The ionophore-sensitive change in fluorescence yield (Fig. 6) is likely associated with a trans-membrane electric field. A calibration of this change against field strength awaits experiments in which fluorescence and photopotentials (-currents) are measured simultaneously in single chloroplasts. These experiments are under consideration to be done.

Extrapolation of the fluorescence decline with increase in time (Fig. 5) will show that the fluorescence yield asymptotically reaches a quasi-stationary level which is 20 to 30% above $F=\mathrm{Fo}$. This is an illustration of an earlier documented effect of preillumination on the dark fluorescence yield as was illustrated in Fig. 4. It has been ascribed to a static electrical effect of accumulated charges accompanying the onset of excitation in a dark-adapted sample on the fluorescence yield [6] and quantifiable using Eq. (3).

It is of interest to compare the efficiencies ϕ_{tr} of charge stabilization (electron trapping) in open and semi-open RCs. By definition

$$\phi_{tr}^{open} = \frac{k_e + k_y}{k_e + k_y + k_{-1} + k_d} \tag{9}$$

and

$$\phi_{tr}^{semi-open} = \frac{k_y}{k_y + k_{-1} + k_d} \tag{10}$$

With the effective rate constants summarized in the table and with $k_{-1} \sim 0.3\ (\mathrm{ns})^{-1}$ [6], one would get $\phi_{tr}^{open}=0.9$ and $\phi_{tr}^{semi-open}=0.55$. The strong dependence of k_y on the S-state with lower values for the higher states [34], in contrast to k_e, causes that the trapping efficiency of semi-open RCs is much lower for the higher S-states and under steady state light conditions. In general, one finds, like in the present intact leaf a manifold lower trapping efficiency in semi-open (-closed) RCs as compared to open RCs. For instance, according to the analysis (not shown, but see simulated curve in Fig. 3), the closing of semi-open centers during the J–I phase occurs in this leaf with an efficiency $\phi_{tr}^{semi-open}=0.03$. This is due to the low rate constant k_y of Y_Z oxidation in S_4 state of donor side. In the present experiment, the analysis gave $k_y\ (S_4) \sim 9\ (\mu s)^{-1}$. The low trapping efficiency of semi-open centers is the major factor determining the relatively slow thermal phase (J–I phase, see Fig. 3) in multi turnover excitations.

Acknowledgement

We thank Richard Poole of Hansatech Ltd. for the loan of the Handy Pea with which part of the experiments have been done.

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