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Photoelectric effects on chlorophyll fluorescence of photosystem II in vivo. Kinetics in the absence and presence of valinomycin

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Abstract

Fluorescence induction curves ($F(t)$) in low intensity 1s light pulses have been measured in leaf discs in the presence and absence of valinomycin (VMC). Addition of VMC causes: (i) no effect on the initial fluorescence level Fo and the initial (O–J) phase of $F(t)$ in the 0.01–1 ms time range. (ii) An approximately 10% decrease in the maximal fluorescence Fm in the light reached at the P level in the O–J–I–P induction curve. (iii) Nearly twofold increase in the rate and extent of the $F(t)$ rise in the J–I phase in the 1–50 ms time range. (iv) A 60–70% decrease in the rise (I–P phase) in the 50–1000 ms time range with no appreciable effect, if at all, on the rate. System analysis of $F(t)$ in terms of rate constants of electron transfer at donor and acceptor sides have been done using the Three State Trapping Model (TSTM). This reveals that VMC causes: (i) no, or very little effect on rate constants of e-transfer reactions powered by PSII. (ii) A manifold lower rate constant of radical pair recombination (k_{-1}) in the light as compared to that in the control. The low rate constant of radical pair recombination in the reaction center (RC) in the presence of VMC is reflected by a substantial increase in the nonzero trapping efficiency in RCs in which the primary quinone acceptor (Q_A) is reduced (semi-open centers). This causes an increase in their rate of closure and in the overall trapping efficiency. Data suggest evidence that membrane chaotropic agents like VMC abolish the stimulation of the rate constant of radical pair recombination by light. This light stimulation that becomes apparent as an increase in Fo has been documented before [Biophys. J. 79 (2000) 26]. It has been ascribed to effects of (changes in) local electric fields in the vicinity of the RC. The decrease of the I–P phase is attributed to a decrease in the photoelectric trans-thylakoid potential in the presence of VMC. Such effects have been hypothesized and illustrated [Bioelectrochemistry 57 (2002) 123].

Keywords: Electric field effect; Chlorophyll fluorescence; Photochemical quenching; Trapping mechanism; Photosystem II; Valinomycin

1. Introduction

The electric field is a natural factor affecting photosynthetic energy conversions in the thylakoid membranes. The initial events of photosynthesis, occurring in a sub-millisecond to second range, proceed under large variations of the transmembrane electric field. The membrane potentials up to 150 mV can be measured with microelectrodes upon photoexcitation [1,2], and they decrease several fold within few seconds during the transition to a steady state. Regulation of photosynthetic electron transport in chloroplast by the transmembrane electric potential is a largely recognized phenomenon [3–5]. The electric field is expected to affect only electrogenic stages of the electron-transport pathway, i.e., the reactions associated with charge separation or recombination. The regulatory effect of the membrane potential on linear electron transport was first revealed with valinomycin (VMC) [3], an agent that increases the potassium permeability of the membranes and substantially suppresses the photogeneration of the membrane potential in chloroplasts [6]. The stimulation of linear electron transport by valinomycin was reported and interpreted as being due to the release of the hindering effect of membrane potential on the electrogenic reaction of plastoquinol oxidation in the cytochrome b_6f complex [3].

Abbreviations: RC, reaction center; PSII, photosystem II; P_{680} (or P), primary electron donor of PSII; Y_Z, secondary electron donor of PSII; Phe, primary electron acceptor of PSII; Q_A, primary quinone acceptor of PSII; ODE, ordinary differential equations; OEC, oxygen evolving complex; VMC, valinomycin; Φpd, quantum yield of PSII photochemistry in dark-adapted leaf samples; $\Phi_{tr}^{(s)o}$, electron trapping efficiency in (semi-) open reaction centers; rFv [=(F−Fo)/(Fm−Fo)], relative variable fluorescence yield.

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Apart form the influence on plastoquinol oxidation, the electric field exerts its effect on recombination of charges in photosystem II (PSII) and PSI [7–11]. These effects were evidenced by stimulatory action of external electric fields on the delayed light emission, which results from recombination of long-lived photoproducts in the donor and acceptor sides of both photosystems. The photochemical reactions involving primary donors and acceptors of PSI and PSII were initially disregarded as possible sites of regulation of electron transfer by the membrane potential, because free energy changes associated with these reaction were considered incomparably large with respect to the electric potential energy. However, the discovery of electric field effects on PSII chlorophyll fluorescence [8,12–14] and the occurrence of reversible stages in the process of energy stabilization [15,16] favoured the possible role of membrane potential in regulation of electron transport in PSII [4,17].

A better understanding of the electric field effects on PSII reactions can be obtained by studying chlorophyll fluorescence transients under conditions when the extent and kinetics of the thylakoid membrane potential is modified by inhibitors, electron transport cofactors and ionophores [18,19]. Valinomycin (VMC) is a useful tool to identify processes controlled by the membrane potential; this ionophore substantially diminishes or modifies the thylakoid membrane potential in the presence of potassium ions. Pospisil and Dau [19] measured the so-called O–J–I–P fluorescence transients [20] in isolated thylakoids treated with VMC and observed substantial changes in fluorescence induction kinetics that were enhanced by the addition of K^+ but were evident even in the absence of K^+. By using a three-exponential approximation of the fluorescence induction in the 10-s time scale, these authors came to the conclusion that the photogenerated membrane potential accounts for the appearance of the J–I stage (symbols designate the initial ‘‘jump’’ and subsequent ‘‘intermediary’’ level of chlorophyll fluorescence). In terms of the reversible pair model, the membrane voltage (lumen positive) shifts the equilibrium between the primary charge separation–recombination reactions in PSII toward the chlorophyll excited state which elevates the excited state lifetime and the fluorescence yield.

A different interpretation of the J–I phase in the induction curve has been introduced recently [21]. This stage was thought to reflect a transition of the primary quinone acceptor from its singly reduced (Q_A^-) to a doubly reduced (Q_A^{2-}) state, which was discussed to occur with low electron trapping efficiency (Φ_{tr}) owing to competitively efficient charge recombination in the primary radical pairs. According to this view, elimination of the delocalized membrane potential is a priori not expected to suppress the J–I phase of the fluorescence induction. A hypothesis was presented that the direct influence of the membrane potential on fluorescence, explained within the framework of a model in which the probability for radical pair recombination is considered to be dependent on strength of the local electric field, is manifest in the I–P transition [17]. This view is consistent with the report that VMC inhibits relatively slow components in the fluorescence induction in thylakoid preparations but stimulates the earlier fluorescence components [2]. However, these experiments were performed under specific experimental conditions ensuring the photogeneration of membrane potential by PSI only. Clearly, the experiments should be extended to allow comparison of functionally competent preparations before and after the suppression of membrane potential. This may clarify the assignment of electric field effects to particular stages of the fluorescence induction.

In this work we investigated the influence of VMC treatment on chlorophyll fluorescence induction in pea leaves exposed to low intensity light. The low light level is known to stimulate the manifestation of the I–P fluorescence rise, which, in our view, is related to the electric field effect [17]. The results show a dual effect of VMC on chlorophyll fluorescence in leaf disks: the fluorescence yield was stimulated by the ionophore during the O–J–I transitions and was suppressed during the I–P transition. It is concluded that suppression of the photoinduced membrane potential effectively stimulates the trapping efficiency of the second electron in the PSII reaction centers by means of decelerating the radical pair recombination.

2. Materials and methods

Pea seeds (cv. Premium and Alfa) were soaked and germinated on moistened filter paper. Seedlings were raised on tap water for about 2 weeks. Fully expanded mature leaves were cut in halves and incubated overnight in vials with 2 ml water containing 5% ethanol (control) or in the same amount of water mixed with 0.1 ml of 10^{-3} M solution of valinomycin in ethanol (treatment). Prior to measurements, each leaf half was cut in three segments. These samples were fixed in clip holders having circular openings of uniform dimensions.

The induction curves of chlorophyll fluorescence were measured with a Plant Efficiency Analyzer (PEA fluorometer, Hansatech Instruments, England) and viewed with a WinPea software. The fluorescence was excited with 2-s pulses of red light (650 nm) emitted with light-emitting diodes. The measurements were performed at irradiances of 12, 30, 60 and 125 W/m^2 (approximately 60–650 μmol/(m^2 s)). Comparatively low intensities were chosen because the amplitude of I–P phase in the fluorescence induction curve is considerably reduced in strong light. Fluorescence data were recorded at a sampling rate of 10 μs and 1 ms per point in the time ranges below and above 1 ms, respectively. The experimental traces represent the averages of three samples each illuminated a single time. Samples were dark-adapted prior to measurements for a period of 20 min.

Experimental fluorescence curves are simulated using a model which describes the fluorescence quenching in rela-

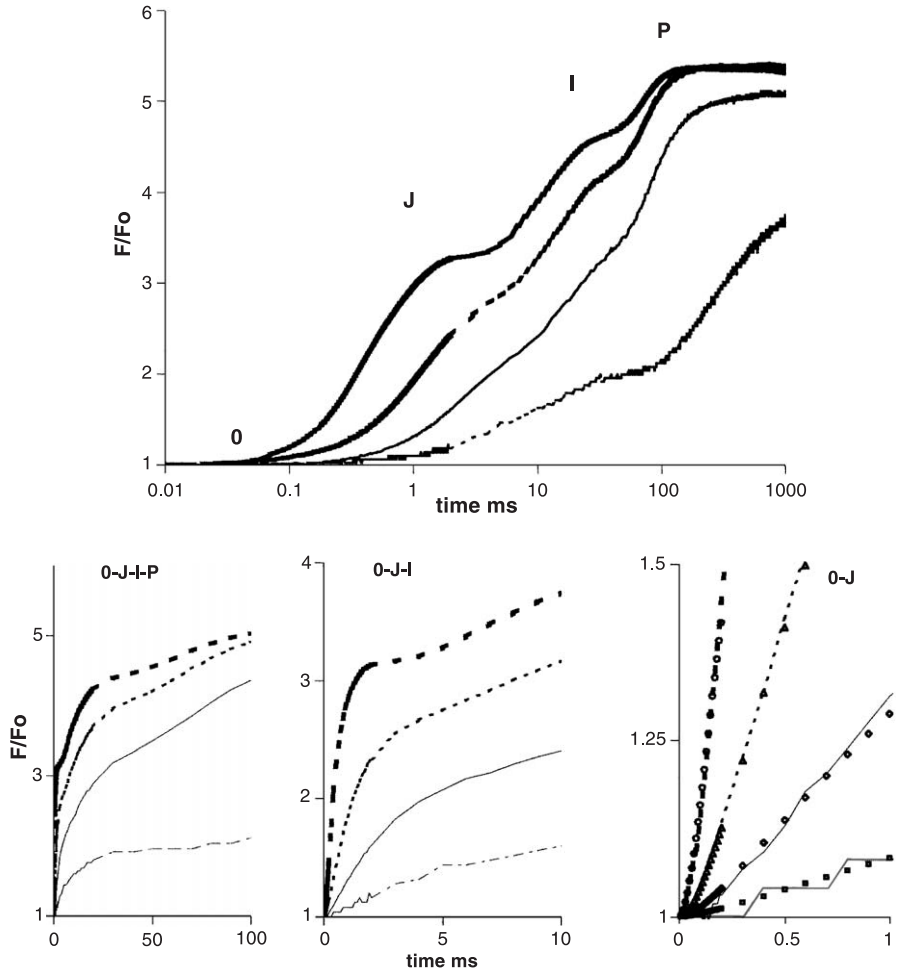

Fig. 1. Kinetics of chlorophyll fluorescence yield, plotted as *F*/Fo, in pea leaf disc in a 2-s light pulse of 12, 30, 60 and 125 W/m^2 intensity (from bottom to top). Upper panel logarithmic time scale and indication of O, J, I and P levels at upper curve. Lower panel linear time scales. Note the difference in scales for both axes. Absolute values at O level (extrapolated to $t = 0.01$ ms) were proportional to intensity. Symbols in the right-hand figure in lower panel are from theoretical fits (see text).

tion to energy trapping in terms of rate constants of primary and associated reactions at the donor and acceptor sides of PSII. Briefly, this model [21,22] accounts for the following trapping properties of PSII: (i) full closure of a RC requires at least two single turnovers (excitations), (ii) efficient fluorescence quenching by positively charged intermediates in the charge transfer pathway between the oxygen evolving complex (OEC) and the special pair (donor side quenching), independent of the negative charges accumulated at the acceptor side (Q_A^-), and (iii) in addition to photochemical quenching, the PSII fluorescence yield is under photoelectrochemical control that is exerted by trans-thylakoid and local electric fields in the vicinity of and sensed by the RC [17]. The model enables the trapping process, i.e. the closure of the RCs 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 optimisation routines (Mathcad 2001i and elder versions, MathSoft, Cambridge, MA, USA) gives the simulation of the experimental curve in terms of reaction rate constants and light excitation rate.

Table 1

I	Control					+ Valinomycin		
	k_L	$k1$	IP	k_v	k_p	IP	k_v	k_p
125	1.8	12	0.9	0.027	0.027	–	–	–
60	0.8	9	1.45	0.024	0.024	–	–	–
30	0.4	8	2.1	0.020	0.020	0.5	0.018	0.01
12	0.18	8	1.9	0.006	0.006	0.7	0.03	0.005

Intensities (W m^{-2}), rate constants (in ms^{-1}) k_L (excitation rate) and $k1$* (release of donor side quenching) of initial fluorescence rise (O–J phase) in a leaf disc are in the three left-hand columns. The initial O–J rise has been approximated, after solving the pertinent ODEs (see Fig. 6 upper row with $\Phi_{tr} = 1$), with (except for an amplitude scaling factor):

$$F_{OJ}(t) = 1 - [k_L/(k_L + k1)]\exp(-k1t) + [k1/(k_L + k1)]\exp(-k_L t) \quad (2)$$

Amplitude (IP) and rate constants (in ms^{-1}) of the fluorescence rise in the light in the 20–1000 ms time domain (I–P phase) in dependence of light intensity and (at $I = 12$ W m^{-2}) in the absence and presence of valinomycin. The rise has been approximated with Eq. (1).

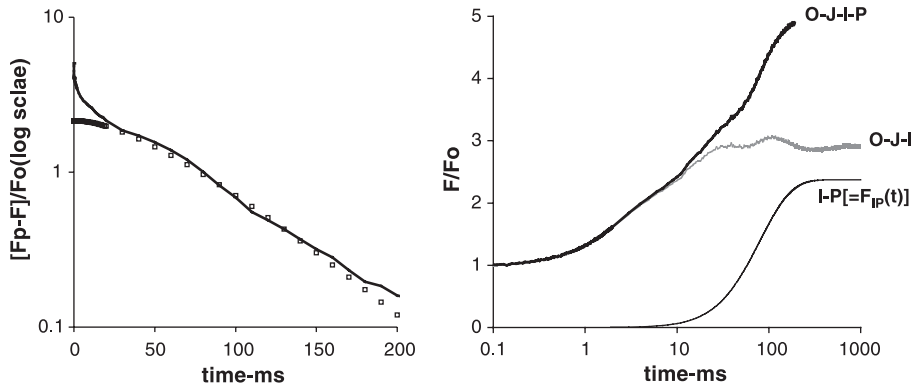

Fig. 2. Chlorophyll fluorescence yield, relative to Fo, plotted as the difference between yield at $t = 500$ ms (F_P) and $F(t)$ (solid curve in the left-hand panel, with log scale) and as F/Fo (right-hand panel, OJIP curve) as function of time. Curve with symbols (left-hand panel) is the biexponential fit ($F_{IP}(t)$, see text) of the experimental curve for $t = 25-175$ ms with IP = 2.14, $k_v = k_p = 2.1\ 10^{-2}$ ms^{-1} (see text). $F_{IP}(t)$ (= IP) is plotted in the right-hand panel. The OJI curve is the difference between full curve (OJIP) and FIP(t). Intensity of light 30 W/m^2.

Valinomycin (Sigma, USA) was dissolved in ethanol solution at a concentration of 10^{-3} M.

3. Results and interpretation

Fig. 1 (upper panel) shows the logarithmic time plot of the fluorescence rise F/Fo of a leaf disc upon 1 s light pulses of 12, 30, 60 and 125 W/m^2 intensity (from bottom to top). The distinct F/Fo levels at $t \sim 0.01$, ~ 1, ~ 20 and ~ 100 ms are designated with O, J, I and P, respectively, following an adopted nomenclature [20]. In the lower panels, the linear time plots of F/Fo (from left to right) are given for the 100, 10 and 1 ms time domain, respectively. The J and I levels decrease and require more time to be reached when the intensity is lower. These levels are not distinguishable at the lowest intensity. In confirmation with other reports [20,23,24], the maximal P level is much less variable with intensity. It hardly changes at intensities between 125 and 30 W/m^2. The decrease in the fluorescence rise in the 0.01–20 ms time domain (O–J–I–rise) apparently is compensated by an increase of the rise in the 20–200 ms time range (I–P rise). At 12 W/m^2, the I–P rise, although still appreciable, is unable to fully compensate the low O–J–I rise. The absolute Fo levels (not shown) were found to be proportional to the intensities that were used. The initial fluorescence rise also was found to be linear with intensity. The rate of this rise is determined by the excitation rate (k_L) and the rate constant ($k1$) of the reaction governing the release of donor side fluorescence quenching [22]. In a first approximation, the O–J rise can be described by a biexponential function $G(t,k_L,k1,\Phi pd)$ in which $\Phi pd = Fv/Fm$ is the quantum yield of PSII photochemistry in a dark-adapted leaf. For the present experiment, $\Phi pd = 0.79$, $k1 \sim 10$ and,

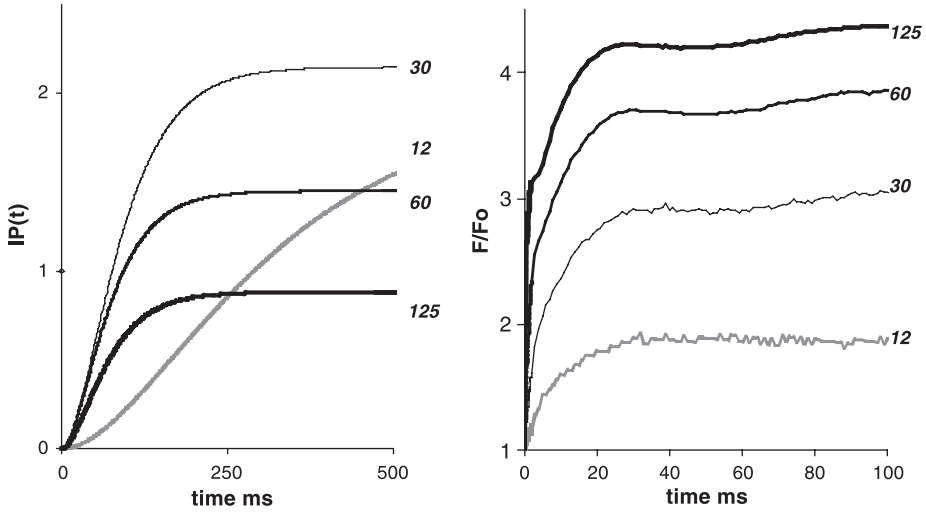

Fig. 3. Time pattern (left-hand panel) of the fluorescence increase (in units relative to Fo) during the final (I–P) phase of the induction in a 1-s light pulse of variable intensity indicated by the number (in W/m^2) at each curve. The right-hand panel gives the fluorescence induction (relative to Fo) of the photochemical O–J–I phase (see text) after subtraction of the photoelectrochemical I–P phase from the overall induction curve (see right-hand panel of Fig. 2).

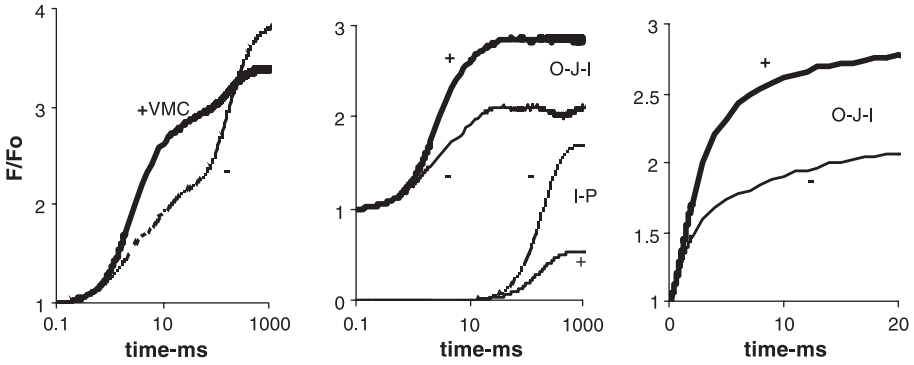

Fig. 4. Kinetics of chlorophyll fluorescence yield, plotted as *F*/Fo, in pea leaf disc in a 2-s light pulse of 12 W/m^2 intensity in the presence (+) and absence (−) of valinomycin (left-hand panel, logarithmic time scale). Deconvoluted O–J–I and I–P curves are shown in the central panel. In the right-hand panel, the O–J–I rise, associated with release of photochemical quenching is shown on a linear time scale.

proportional with intensity, k_L variable from ~ 0.2 to 1.8 ms^{-1} as shown in Table 1. The calculated rise curves are presented in the right-hand lower panel.

Fig. 2 shows the result of the decomposition of the *F*/Fo curve into the O–J–I and I–P phases. As shown by others [19], the I–P phase in the overall curve (Fig. 1) can be approximated by an exponential function. The left-hand panel shows the log plot of $[F_P - F(t)]$/Fo vs. time for the O–J–I–P curve at 30 W/m^2. The linear relation at t>50 ms indicates and confirms this exponential character. The figure shows the fit of the I–P phase with a biexponential function to allow for the curvature in the 25–50 ms time domain.

$$F_{IP}(t) = IP\{[k_v/(k_v + k_p)]\exp(-k_p t) - [k_p/(k_v + k_p)]\exp(-k_v t)\} \quad (1)$$

in which IP is the amplitude of the I–P phase and k_v and k_p are rate constants with subscripts v and p referring to possible relation with generation and propagation, respectively, of the photocurrents. The O–J–I phase is obtained by subtracting $F_{IP}(t)$ from the overall curve (right-hand panel of Fig. 2).

Fig. 3 shows the O–J–I and I–P curves for the separate intensities that were used. It illustrates the decrease and increase of O–J–I and I–P phase, respectively, upon lowering the intensity.

Fig. 4 shows the *F*/Fo curve of a leaf disc before and after treatment with valinomycin (VMC) in a 12 W/m^2 light pulse. The central panel shows the deconvoluted O–J–I and I–P phases. VMC causes (i) an approximately 15% lowering of the P level and no change in the O level (Fo), (ii) an approximately 65% increase in the O–J–I rise concomitant with a nearly complimentary decrease in the I–P rise and, as shown for the O–J–I rise in the right-hand panel, (iii) little effect on the initial rates of the O–J–I and the I–P fluorescence rise.

Fig. 5 shows the simulation of the experimental fluorescence curve in the absence and presence of VMC. It has

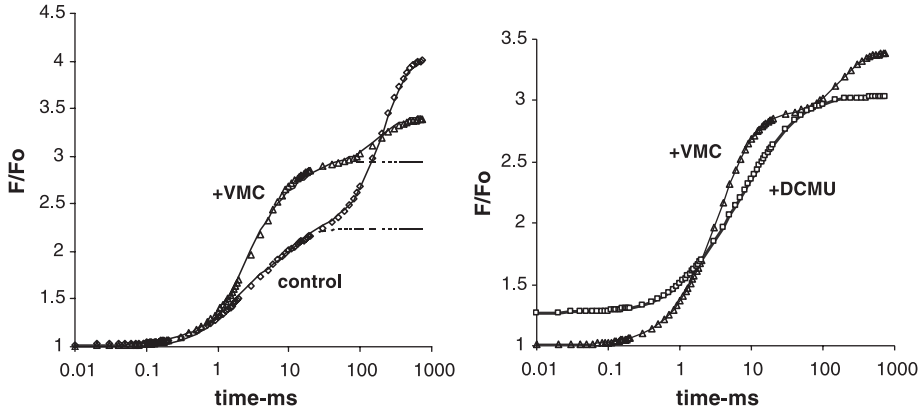

Fig. 5. Experimental (solid lines) and simulated (symbols) kinetics of chlorophyll fluorescence yield, plotted as *F*/Fo, in pea leaf disc in a 2-s light pulse of 12 W/m^2 intensity in the presence (triangles) and absence (diamonds) of valinomycin (left-hand panel). Simulation was made by biexponential fit of the curves in the 20–2000 ms time domain (I–P phase) and by parameter optimization after solving the multi-set of differential equations underlying a double hit trapping model and emphasizing the donor-side fluorescence quenching beyond P^+ to be controlled by the rate constant of radical pair recombination. The effect of valinomycin on fluorescence then would suggest that the ionophore keeps this rate constant low in the light. Horizontal lines in the 20–1000 ms time range mark the steady state I levels in the simulation. The right-hand panel shows simulations of the fluorescence kinetics in the presence of VMC and DCMU, illustrating the distinct difference of these compounds on the fluorescence induction curve.

Table 2

	k_L	$k1$	$k2$	$k3$	$k4$	k_{y1}	k_{y2}	k_{y3}	k_{y4}	kAB1	kAB2	kPQ	k_{-1}	β
Control	0.45	9.8	4.1	0.95	27	2×10^5	7×10^4	2×10^4	1×10^4	0.26	0.08	0.035	3×10^5	0.25
+VMC	0.38									0.11	0.04		2×10^2	
+DCMU	0.41									0.00	0.00		3×10^5	

Fit parameters (rate constants, in ms^{-1}) of the fluorescence rise in the light in the 0.01–20 ms time domain (O–J–I rise) in the absence and presence of valinomycin or of DCMU (see Fig. 5).
k_L: excitation rate; $k(i)$ and $k_{y(i)}$: reduction of Y_Z^+ and P^+, respectively, with OEC in state S(i) (i = 1,. . .,4); kAB1(2): single and double reduction of Q_B by Q_A^-; kPQ: pseudo-first-order rate constant for reoxidation of Q_BH_2 by plastoquinone pool. β: fraction of RCs in S0 state and with Q_B singly reduced.

been assumed that the fluorescence rise in the 0–20 (O–J–I) and 20–1000 ms time domain (I–P) is associated with (release of) photochemical quenching and photoelectrochemical stimulation, respectively [17]. Furthermore, that photochemical quenching is governed by a double hit, so-called three-state trapping model (TSTM). The I–P phase is obtained after fitting the 'tail' of the experimental curve with a biexponential function (see before). The fit parameters of the photochemical O–J–I phase are given in Table 2. It is clear that the fit parameters of the curves in the presence and absence VMC are hardly different, if at all, except for the rate constant of radical pair recombination (k_{-1}) which is manifold lower in the presence of the ionophore. The effect of DCMU on $F(t)$ is, for comparison, illustrated in Fig. 5 (right-hand panel). It shows a distinct difference with that of VMC.

4. Discussion

The present data show and confirm earlier results that the ionophore valinomycin in the presence of K^+ ions has a pronounced effect on the kinetics of the multiphasic rise in chlorophyll fluorescence yield in a light pulse of prolonged duration [18,19]. VMC causes, as Fig. 4 illustrates, a substantial increase in the light-induced fluorescence transient in the 1–20 ms time domain. In addition, it causes a decrease of the maximal fluorescence level in the light at about 500 ms (P level) and of the rise in the light in the 20–500 ms time region (I–P phase). Finally, there is neither an effect of the ionophore on the initial fluorescence level Fo, nor on the initial rate of the fluorescence increase at the onset of illumination in the 0.01–1 ms time domain. These distinct effects allow some qualitative interpretations in terms of current trapping models [19] and a recently proposed hypothesis on photochemical and photoelectrochemical fluorescence quenching [17,18].

4.1. Photoelectrochemical stimulation of PSII fluorescence: I–P phase

The much lower sensitivity of the maximal fluorescence yield in the light above 500 ms (P level) to light intensity as compared to that at 2 and 20 ms (J and I levels, respectively), confirming earlier reports [20,23] and the nearly complete compensation of the decrease of the O–J–I phase by an increase in the I–P phase gives support for the hypothesis that the latter is associated with a photoelectrochemical effect. As discussed and illustrated extensively in a recent paper [17], there is theoretical evidence that the yield of PSII fluorescence is under photochemical and photoelectrochemical control. Increase in fluorescence yield associated with release of photochemical quenching due to RC closure is complemented with increase in yield associated with effects of electric field strength on the excited state of the RC chlorophyll and radical pair recombination in the RC. This effect is likely to be exerted by fields originating from the photoelectric trans-thylakoid potential or from local single charges or dipoles. A prominent contribution of the photoelectric potential generated by PSI and transversally propagated to the PSII active sites to the PSII fluorescence yield has been demonstrated [18]. The biexponential kinetics of the I–P fluorescence phase in the 20–1000 ms time region (Fig. 2; Table 1) might suggest a substantial contribution of PSI potential generation to PSII fluorescence emission at lower intensities, at which RC closure by PSII becomes light limited. In this respect the low sensitivity of the parameter k_p to light intensity (Table 1) might point to its association with the rate of lateral propagation of the potential generated by PSI through the conducting stroma sheets in the thylakoids. A close relation between the kinetics of I–P fluorescence increase and changes in lateral conductance under dominant PSI light activation has been demonstrated [18]. The clear effect of VMC in lowering the I–P fluorescence phase is consistent with an association of this phase with electric fields. The nonproportional effect of VMC on amplitude and rate constants of the I–P phase at the intensities used (see Table 1) would be consistent with a distant generating and sensing site of the photoelectrochemical effect.

4.2. The rate of initial fluorescence rise from Fo: O–J phase

The initial fluorescence level Fo and the rate at which this yield rises in the light in the 0.01–1 ms time range was found to be linearly related to light intensity under our experimental conditions. Fo reflects the fluorescence yield at full photochemical quenching in the absence of photoelectrochemical stimulation (i.e. under dark-adapted conditions). Neither Fo nor the rate of the initial rise is affected after addition of valinomycin. This indicates that the target

site of the ionophore, which has a noticeable effect in the time region above 1 ms, is not at the RC site(s) at which primary trapping and charge separation occurs and likely also not at the organizational antenna size level. In this respect, the effect of valinomycin is quite different from that of triazine- and phenylurea-type herbicides, as illustrated for DCMU in Fig. 5. These agents cause changes in Fo and in the rate of the initial fluorescence rise, as will be reported in a forthcoming communication (Hiraki et al., in preparation, but see also Refs. [21,22]).

The insensitivity of Fo and the rate of the initial rise to VMC, in contrast to substantial effects after the initial events (Figs. 4 and 5), is of prime importance and requires attention in particular with respect to the trapping mechanism which determines photochemical quenching of fluorescence emission.

4.3. Fluorescence rise in the 0.01–10 ms time domain: O–J–I rise

The rate at which the fluorescence rises in the light in the 1–20 ms time region is considerably increased in the presence of VMC, in contrast to an unaltered initial rise during the first 1 ms. This points to a mechanism in which trapping during a (the) first turnover(s) occurs in a different environment than in subsequent turnovers. The supposed difference might be (i) an altered concentration or activity of fluorescence quenchers, (ii) an altered trapping property of the RC, or (iii) a combination of both. The first possibility is in harmony with models and views advocated by others [20,25,26], except for the fact that the quencher apparently has to be generated or activated by (a product of) the first turnover(s) to allow for the difference in quenching behaviour in first and following turnovers. The effect of VMC then would point to an enhancement of the release of the quenching, or enhanced conversion of the quencher. However, to our knowledge, none of the data and interpretation models published so far has offered evidence for the generation and existence of this type of quencher. In addition, none of the quenchers identified so far, i.e. Q_A, $P680^+$, car^T, PQ, has been reported to possess the property to behave differently in first and subsequent turnovers.

The second possibility of an altered trapping property of the RC after a first turnover finds support in the recently proposed Three-State Trapping Model of PSII [21,22]. This model is based on a double hit trapping mechanism and discriminates between open, semi-closed (-open) and closed RCs with a relative variable fluorescence yield (rFv) equal to rFv = 0, 0.5 and 1, associated with 0, 1 and 2 electrons trapped at the acceptor side, respectively. It has been argued that during its life time a semi-closed RC, formed with high trapping efficiency in a first hit (turnover), can be transferred into a closed RC upon a second hit. This transfer however occurs with a low efficiency and is under control of the rate of radical pair recombination in the RC, as we will briefly illustrate now (Fig. 6).

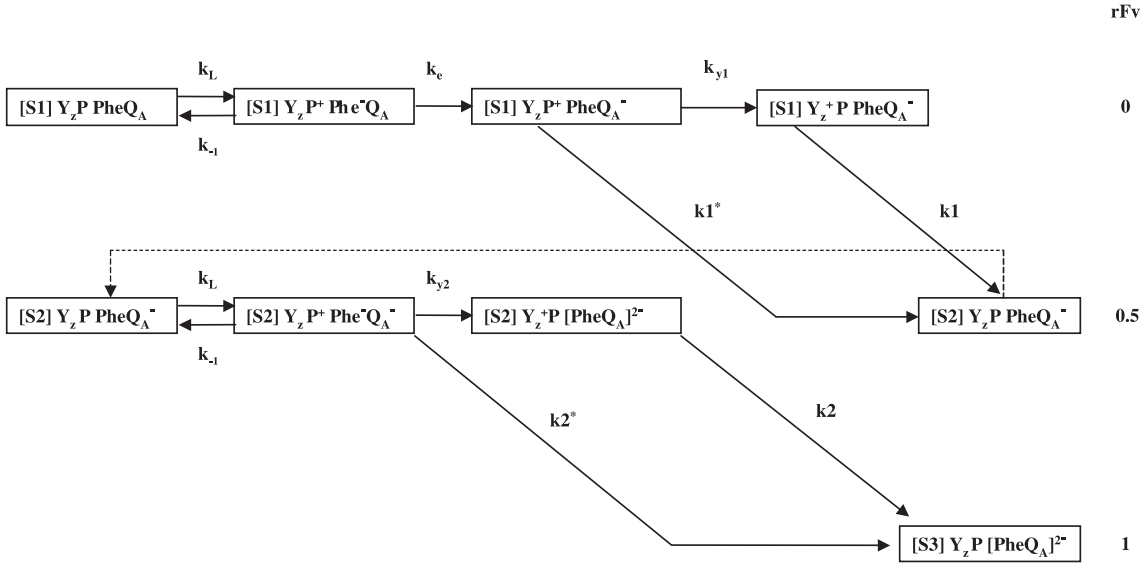

Fig. 6. Simplified representation of reaction patterns and intermediates of the transfer of an open (upper row) and semi-closed reaction center (central row) into a semi-closed (central) and closed form (bottom row), respectively, in a light pulse with excitation rate k_L. The oxygen evolving complex (OEC) of dark-adapted open RC [S1] $Y_Z P\ PheQ_A$ is assumed to be in state S1. Further simplifications are (i) initial excitation and charge separation, and recombination steps are combined, (ii) low efficiencies for losses different form photochemical storage (heat, nonradiative charge recombination, ^{T}RC formation) are assumed, and (iii) electron flow beyond Q_A^- is for the ease of explanation (see text) and illustration not considered. The location of the 2nd electron trapped at the acceptor side is not specified, and designated with $[PheQ_A]^{2-}$. A net positive charge (+) in the transfer pathway between OEC and RC is assumed to cause photochemical quenching. The release of this quenching is designated to occur with rate constants $k1^*$ and $k2^*$. Rate constants k_e, $k_{y1(2)}$ and $k1(2)$ are rate constants for the reduction of Q_A, P^+ and Y_Z^+, respectively, with OEC in S1 (or S2) state. Values of relative variable fluorescence yields (rFv) for RC states in each of the three rows are given in the right-hand column.

4.4. The fluorescence rise in the 1–20 ms time interval ([O–]JI phase) and radical pair recombination: effect of VMC

A first turnover excitation of an open reaction center [S1] Y_ZP $PheQ_A$ which, with $k_L \sim 1$ ms^{-1}, will occur in the initial time domain between 0.01 and 1 ms, leads to the semi-closed state of the RC [S2] Y_ZP $PheQ_A^-$. The reaction pattern is shown in the upper row of Fig. 6 in which k_L and k_{-1} are rate constants of RC excitation and radical pair recombination, respectively, and k_e, k_{y1} and $k1$ are those of Q_A, P^+ and Y_Z^+ reductions with the OEC in state S1. The trapping efficiency in an open RC (Φ_{tr}^o) for trapping the electron in Q_A is $\Phi_{tr}^o = k_e/(k_e + k_{-1})$. Because in general, $k_e > k_{-1}$, the efficiency in an open center, is close to 1. This reaction pattern can be simplified by taking k_{y1} and $k1$ collectively together and replacing it with $k1^*$. This constant can be attributed to the rate constant for the release of the donor side quenching exerted by positively charged intermediates in the electron transport pathway between OEC and the special pair (see Fig. 6, 1st row).

Excitation of the semi-closed RC [S2] Y_ZP $PheQ_A^-$, initiates the cascade of reactions resulting in full closure of the RC, as illustrated in the 2nd row of Fig. 6. The efficiency with which electron trapping occurs in a semi closed state like [S2]Y_ZP $PheQ_A^-$ is $\Phi_{tr}^{so} = k_{y2}/(k_{y2} + k_{-1})$. According to reported values for k_{y2} and k_{-1}, both of the order of about (10 ns)$^{-1}$ and S-state-dependent [27–29], Φ_{tr}^{so} is much less than 1, but nevertheless nonzero, in contrast to what generally is assumed in current trapping models [24–26,30,31]. The low electron trapping efficiency in semi-open centers causes a low actual rate of RC closing. It is reflected by the slow fluorescence rise (J–I phase) in the 2–200 ms time region (Figs. 1 and 3). An increase in the actual rate is predicted to occur concomitant with an increase in the trapping efficiency Φ_{tr}^{so}. Φ_{tr}^{so} will increase concomitant with either an increase in k_{y2}, or a decrease in k_{-1}. The effect of VMC in causing an increased rate of the release of the fluorescence quenching in the J–I phase of the induction period in the light would be conclusive with an effect on the efficiency with which electron trapping in singly reduced semi-open RCs occurs.

4.5. Simulation of the O–J–I–P fluorescence induction curve in terms of PSII rate constants: effect of VMC

The analysis and simulation of the full O–J–I–P induction curve in the presence and absence of VMC (Fig. 5; Table 2) indeed shows that the major, if not exclusive effect of valinomycin on the primary reactions of PSII is on the rate of radical pair recombination in the light. A reduction with a factor 10^3 from $k_{-1} \sim 10^5$ to 10^2 ms^{-1} with only marginal changes, if at all in the other rate constants, gives a good fit for the $F(t)$ curves in the absence and presence of VMC. It should be noted that from a kinetic point of view the rate constants in the light for Y_Z^+ reduction ($k_{y1(2)}$) could have been increased with the same factor instead of the decrease in k_{-1}. However, this would have given unlikely high values for these rate constants. A much lower value than $k_{-1} \sim 10^5$ ms^{-1}, which has been reported for light conditions [29] has been concluded to exist in the dark [21]. The dark fluorescence yield under maximal photochemical quenching (Fo) was shown to be associated with a rate constant of radical pair recombination in the RC that was much lower than after pre-illumination even with a single turnover flash. The magnitude of the 15–25% increase in Fo after single flash excitation has quantitatively been shown to be consistent with a more than 100-fold increase in k_{-1} towards a value of approximately 10^5 ms^{-1} and stable for a dark period of minutes [21].

It is tempting to conclude from the present experiments that VMC inhibits the increase of the rate constant of radical pair recombination of the RC in the light. The documented light effect on the rate (constant) of radical pair combination in the absence of the ionophore has been suggested to be associated with an electrostatic effect of local charges, presumably those accumulating in the OEC at the donor side, on the quasi-equilibrium between energetic states of excited RC chlorophyll and of the radical pair P^+Phe^-. This effect could well be associated with the chaotropic properties of the ionophore. Further experiments are required to study the reported effect of VMC in relation to its activity as an ADRY agent and to test whether or not other ADRY agents [32] share this property in interacting with the energetics of excited and charge-separated states within the photosynthetic reaction center of PSII.

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