LETTER TO THE EDITOR

Kinetic models of photosystem II should accommodate the effect of donor side quenching on variable chlorophyll *a* fluorescence in the microseconds time range

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Abstract Quantitative data on laser flash-induced variable fluorescence in the 100 ns to 1 ms time range (Belyaeva et al. in Photosynth Res 98:105–119, 2008) confirming those of others (Steffen et al. in Biochemistry 40:173–180, 2001, Biochemistry 44:3123–3132, 2005; Belyaeva et al. in Biophysics 51(6):976–990, 2006), need a substantial correction with respect to magnitude of the normalized variable fluorescence associated with single turnover-induced charge separation in RCs of PS II. Their data are conclusive with the involvement of donor side quenching, the release of which occurs with a rate constant in the range of tens of ms^{-1}, and presumed to be associated with reduction of Y_Z^+ by the OEC.

Keywords Chlorophyll *a* fluorescence · PS II modeling · Donor side quenching

Measurement of chlorophyll (Chl) fluorescence with high time resolution is a non-invasive method, which offers a valuable tool for quantitative analyses of the dynamics and time patterns of the primary processes in photosystem II (PS II) of photosynthesis. In a recent paper "PS II model-based simulations of single turnover flash-induced transients of fluorescence yield monitored within the time domain of 100 ns–10 s on dark-adapted Chlorella pyrenoidosa cells" (Belyaeva et al. 2008). Natalia Belyaeva et al. from Andrew Rubin's and Gernot Renger's groups have shown impressive results of a quantitative analysis of

the chlorophyll fluorescence transients in a time domain that covers eight decades.

Their paper raises, however, a problem with respect to the magnitude of the variable fluorescence F_v^{STF} ($=F_m^{STF} - F_o$) that is associated with a single turnover of PS II which comprises charge separation and stabilization in its reaction center (RC). F_o is the initial dark fluorescence level and minimal due to full photochemical quenching of fluorescence emission in antennas of so-called open RCs; F_m^{STF} is the maximal fluorescence of so-called semi-closed RCs which all have made one turnover and an electron trapped at the secondary acceptor Q_A and the positive charge at the donor side beyond the primary donor P680. The single turnover-induced formation of Q_A^-(Q_A − reduction) has caused an increase in fluorescence emission due to the release of photochemical quenching by Q_A. Usually time responses of fluorescence emission $F(t)$ in the light are plotted relative to F_o.

$F(t)/F_o$ data in Chlorella (Belyaeva et al. 2008, see Figs. 2, 3) show, in agreement with those reported by Ronald Steffen et al. for other species, that the maximum of the normalized variable fluorescence nF_v^{STF}($=[F_m^{STF} - F_o]/F_o$) upon a saturating 10 ns laser flash is reached in the time range between 10 and 100 μs with $0.8 < nF_v^{STF} < 1$. Values of nF_v^{STF} in this range are at variance with and 50% below $nF_v^{STF} \sim 2$ reported for a variety of organisms and routinely measured with flashes of 30 μs duration in a Dual-Modulation Kinetic Fluorometer (PSI, Brno, Cz). These 30 μs-flashes can be considered as STFs under the conditions used. Moreover, it has been reported that double (TTF) and multiple excitations with these STFs causes a relatively small and transient increase in nF_v^{STF} ascribed to quenching release associated with electron trapping in reduced Q_B-nonreducing (semi-open) RCs (Vredenberg et al. 2007).

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If one would accept $nF_v^{STF} = 1$ from Belyaeva's model and experiments, it would mean that the release of photochemical quenching (Q_A reduction) has to be supplemented with an approximate threefold higher release of fluorescence quenching from other origin, in order to accommodate $nF_v^{STF} \sim 4$ in multi-turnover light pulses (MTF-excitation). The latter value corresponds with $F_v^{MTF}/F_m^{MTF} \sim 0.8$, which is a common and 'proper' value for healthy preparations. It is difficult to imagine that the candidates for this formidable quenching job that are mentioned in their paper can do it.

In addition, the kinetic pattern of the decay in the 100 μs to 10 s time range suggests that, according to size and pattern of the decay in the time range above 20 ms, re-oxidation of Q_A^- in $\sim 50\%$ of RCs occurs in a time above 20 ms. One would expect such high fraction of RCs with low turnover rate of PS II only in preparations with attenuated photosynthetic efficiency. However, the decay patterns presented in Figs. 2 and 3 of the referred paper are also at variance with those reported by other research groups. These routinely show that the fraction with slow decay in the time range above 10 ms is 10–30% of the total RCs and has been attributed to that of Q_B-nonreducing RCs (Vredenberg et al. 2006).

Size and kinetic pattern of the $F(t)/F_o$ response are determined by the rate constants of the release of fluorescence quenching by the (dark) oxidized primary acceptor pair pheophytin (Phe) and Q_A and by (photo-) oxidized intermediates in the PS II donor side electron transfer pathway (Vredenberg 2008). Specifically it has to be considered that the kinetics of laser-induced fluorescence changes in the 1–200 μs time range are determined (i) by the rate constant(s) of the fluorescence increase due to release of donor side quenching (DSQ) and (ii) by that of the fluorescence decrease due the recovery of fluorescence quenching associated with the re-oxidation of Q_A^- at the acceptor side.

Briefly, a non-quenching condition (or state) of RCs with Q_A^- and life time ($1/k_{AB}$) in the range between 150 and 500 μs is formed with rate constant (k_e) of the order of 10^6 ms^{-1} (Belyaeva et al. 2008; Vredenberg 2008). The rate of quenching release is substantially attenuated with respect to k_e and is determined by the rate constant of DSQ-release, which we might call k_{dsq}. It follows that the normalized fluorescence response $F(t)/F_o$ in this simplified concept with 100% Q_B-reducing RCs can be approximated by the relation

$$\frac{F(t)}{F_o} = 1 + nF_v^{STF}(1 - e^{-k_{dsq}t})e^{-k_{AB}t} \tag{1}$$

in which, nF_v^{STF} is the normalized variable fluorescence associated with STF excitation (see for an extensive derivation and explanation Vredenberg and Prasil 2009).

For Q_B-nonreducing RCs k_{AB} in Eq. 1 is replaced by k_{-nqb} where $k_{-nqb} \ll k_{AB}$ is the approximate average rate constant of the slow re-appearance of quenching associated with recovery of these RCs. For a heterogeneous system with a β-fraction (S_0) of Q_B-nonreducing RCs, Eq. 1 can be rewritten with

$$
\begin{aligned}
F^{DSQ}(t) &= \frac{F(t)}{F_o} \\
&= 1 + nF_v^{STF}[(1 - e^{-k_{dsq}t})\{(1 - \beta)^* e^{-k_{AB}t} \\
&\quad + \beta^* e^{-k_{-nqb}t}\}].
\end{aligned}
\tag{1a}
$$

Ample evidence has been given that $nF_v^{STF} \sim 2$ in leaves and thylakoids. This value, with according to definition nF_v^{STF} (=$F_m^{MTF}/F_o - 1$) $\sim 2nF_v^{STF} \sim 4$, corresponds with $F_v^{MTF}/F_v^{STF} \sim 0.8$, which is the 'proper' value for healthy preparations. Under conditions at which $k_{AB} \ll 0.1$ ms^{-1} which is true for Q_B-nonreducing RCs or in the presence of DCMU, the graph of Eqs. 1 and 1a will show an exponential rise with reaction time $1/k_{dsq}$ toward a maximum with $F(t)/F_o = 1 + nF_v^{STF} \sim 3$. This level will also be reached under conditions at which $k_{dsq} \ll k_{AB}$. In this context, it is noteworthy that in the papers of Belyaeva (2006, 2008) and of Steffen et al. (2001, 2005), the maximum $F(t)/F_o$ values are around 1.9. The significantly reduced level of maximal variable fluorescence after laser flash excitation could be due to (i) either a poor quality of the preparations or (ii) to the rate constant k_{dsq} of DSQ release when this is less than 2 orders of magnitude smaller than that of Q_A^- re-oxidation (k_{AB}). A closer analysis, using Eqs. 1 and 1a, will point to evidence for the second interpretation.

Figure 1, with experimental data (closed black diamonds) reproduced from Steffen (Steffen et al. 2005, see Fig. 2 therein), and of similar shape as that reported by Belyaeva et al. (2006, 2008) will serve a further explanation and illustration. The best fit (solid red line) for $F^{DSQ}(t) = F(t)/F_o$ shows (i) a rise from 1 (at 100 ns) to ~ 1.9 reached at $t \sim 20$ μs, and (ii) the well documented biphasic decay with fast (F) phase in the 0.02–1 ms time range towards an intermediate plateau level F^{pl} at $F^{DSQ}(t) \sim 1.3$ followed by the slow (S) phase far into the tens of seconds time range. We have assumed the following parameter values which are in the range commonly found in thylakoids and intact leaves: normalized variable fluorescence in STF, $nF_v^{STF} = 1.8$, rate constants (in ms^{-1}) for DSQ release (k_{dsq}), Q_A^- re-oxidation (k_{AB}), and quenching recovery in double reduced Q_B-nonreducing RCs (k_{-nqb}) 35, 10, and 0.025, respectively, and fraction of Q_B-nonreducing RCs β (=F^{pl}/nF_v^{STF}) $\sim 18\%$. After substitution in Eq. 1a one obtains the simulated time responses of $F^{DSQ}(t)$. The rough simulation, illustrated in Fig. 1 and based on a simplified reaction scheme, shows a reasonable correspondence of the simulation with experimental curve (Steffen et al. 2005, Fig. 2), and a substantial attenuation of
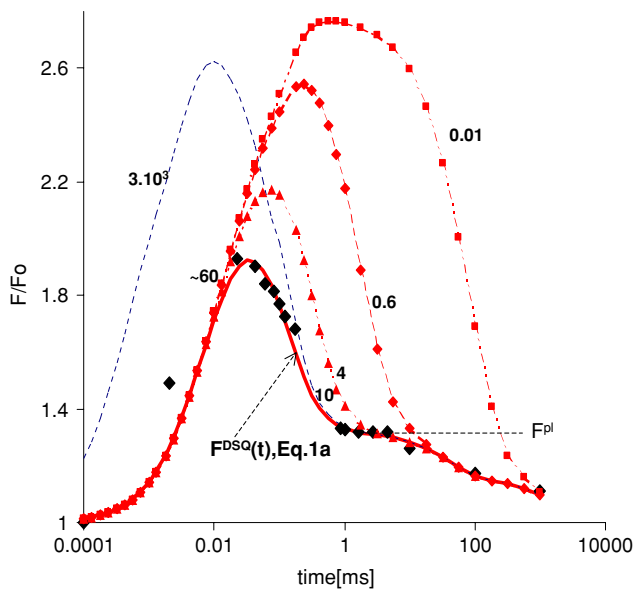
Fig. 1 Relative chlorophyll *a* fluorescence change (*closed black diamonds*) $F(t)/F_o$ of 1 h dark-adapted *Arabidopsis thaliana* leaf in 100 ns to 10 s time range (logarithmic) upon saturating laser flash (6.2×10^{15} photons cm^{-2}/flash), reproduced from Fig. 2 in Steffen et al. (2005). *Bold red curve* is the simulated response $F^{DSQ}(t)$ using a modification of Eq. 1a. The modification accounts for a S_0 (β):S_1:S_2 heterogeneity of 0.2:0.4:0.4 with corresponding rate constants of donor side quenching $k_{dsq} = 300$, 60, and 7 ms^{-1}, $k_{AB} \sim 9$ ms^{-1} and a biphasic decay of Q_B-nonreducing RCs with rate constants $k_{-nqb} \sim 25$ and 0.5 s^{-1} and n$F_v = 1.8$. Note that F^{pl} is from (reduced) Q_B-nonreducing RCs at the fractional size $\beta \sim 0.3/1.8 \sim 18\%$. The red dashed curves (*closed triangles*, *diamonds and squares*) are simulations with variable rate constant of quenching recovery (k_{AB}) due to Q_A^- reoxidation. Parameter values of variable quenching-regeneration (k_{AB}) are indicated at the right-hand side of the respective curves. The *blue-colored dashed curve* shows the $F^{DSQ}(t)$ response when, at constant k_{AB} (~ 10 ms^{-1}), k_{dsq} is increased 50-fold (for instance when donor side quenching (DSQ) is ignored). The *dashed curves* illustrate the effect of interference between k_{dsq} and k_{AB} on the maximum of $F(t)/F_o$ with an increasing disproportion between nF_v^{STF} and the maximum of $F^{DSQ}(t)$ with the increase in rate (k_{AB}) of quenching recovery

the maximum in the $F(t)/F_o$ curve with respect to n$F_v^{STF} = 1.8$. The attenuation decreases with a decrease in k_{AB}, i.e. with attenuation of electron transport at the acceptor side of PS II. This indicates that the low $F(t)/F_o$ maximum in the experimental curve is due to the interference of the rate constants DSQ release and of Q_A^- reoxidation (recovery of quenching). Similarly one can show that the $F(t)/F_o$ response changes (blue solid curve) when the rate constant of the release of DSQ is assumed to be 50-fold higher with $k_{dsq} \sim 15$ μs^{-1}, which would mean the ignorance of DSQ release in a time domain above ~ 10 μs.

In summary, the quantitative data on laser flash-induced variable fluorescence from the 100 ns to 1 ms time range (Belyaeva et al. 2008) confirming those of others (Steffen et al. 2001, 2005; Belyaeva et al. 2006), need a substantial correction with respect to magnitude of the normalized variable fluorescence associated with single turnover-induced charge separation in RCs of PS II. Their data are conclusive with the involvement of donor side quenching, the release of which occurs with a rate constant in the range of tens of ms^{-1}, and presumed to be associated with reduction of Y_z^+ by the OEC.

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