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Importance of the oxidised plastoquinone molecules on the thermal phase of chlorophyll-*a* fluorescence

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Introduction

In photosynthetic organisms, the emission of variable Chl*a* fluorescence is composed of two distinct parts, the photochemical and thermal phases (Samson *et al.*, 1999). The photochemical phase, which represents about 50% of the total variable fluorescence in dark-adapted intact samples, is related to the reduction of PSII primary quinone electron acceptor Q_A. It can be induced by single turnover flash (STF) or by a strong continuous illumination under which the photoreduction rate of PSII electron acceptors exceeds several times its re-oxidation rate. On the other hand, the thermal phase requires multiple PSII turnovers and it corresponds to the slower phase of fluorescence kinetics induced by intense illumination (Neubauer and Schreiber, 1987). It was recently showed that the low yield of Chl*a* fluorescence induced by STF is due to a non-photochemical quenching localised in the PSII antenna (Vasil'ev and Bruce, 1999). One of the most plausible cause of this non-photochemical quenching is the oxidised PQ molecules. Since the quenching effect of the PQ pool on the steady state Chl*a* fluorescence level observed in presence of DCMU generally accounts for only 10% of Fm (Vernotte *et al.*, 1979), it was suggested that the presence of an oxidised PQ molecule bound at the Q_B site of PSII could also be responsible for the quenching corresponding to the thermal phase (Samson *et al.*, 1999). In this context, the main objective of this study was to verify the relative contributions of the free oxidised PQ molecules and those bound to the Q_B site to the thermal phase of Chl*a* fluorescence.

Materials and methods

Spinach thylakoids were isolated as described in Whitmarsh and Ort (1984) from intact leaves sampled on 6 to 8 week-old greenhouse plants. Before each measurement, thylakoids were resuspended (30 μM Chl*a*+*b*) in a medium composed of 0.1 M sorbitol , 50 mM Hepes pH 7.5/NaOH, 5 mM $MgCl_2$, 10 mM KCl, 0.5 mg/ml BSA and then kept in darkness for 3 min. Chl*a* fluorescence was measured at room temperature using a PAM-101/103 fluorometer (Waltz, Effeltrich, Germany) connected to an acquisition card and software (Q_A-Data, Turku, Finland). The photochemical phase of Chl*a* was induced by short saturating single turnover flashes (STF) provided by a XE/XST-103 xenon-flash lamp. The fluorescence traces were averaged from 8 STF given at a frequency of 0.125 Hz. The amplitude of the photochemical phase (Ff level) was measured 150 μs after the STF. Following the 8 STF, the maximum fluorescence level (Fm) was induced by a 1 s saturating flash provided by a FL-103/E fibre illuminator (Waltz, Effeltrich, Germany). Also,

complementary areas were determined from fluorescence induction kinetics recorded with a 'Plant Efficiency Analyser' fluorometer (Hansatech, Norfolk, U.K.).

Results and Discussion

Effects of exogenous decyl-plastoquinone on Chla fluorescence

The incorporation of exogenous decyl-plastoquinone (PQex) into thylakoids was first verified by measuring the complementary area (CA) of Chl*a* fluorescence as a function of added PQex concentration. The CA corresponds to the surface above the fluorescence induction curve and is related to the pool size of electron acceptors, primarily PQ molecules (Lavorel *et al.*, 1986). Fig.1 shows that the CA increased linearly as the PQex concentration increased from 0 up to 20 PQex per PSII reaction centre, assuming 400 Chl*a+b* molecules per PSII. When the initial value of the CA measured in absence of PQex was normalised to one, the slope of the regression was equal to one unit of CA for 10 PQex per PSII, i.e. approximately the ratio of endogenous PQ pool per PSII in spinach thylakoids (Melis and Brown, 1980). These results indicate that most of the PQex were successfully incorporated into spinach thylakoids and acted as efficient electron acceptor. Despite their incorporation, PQex had no or limited effects on the fluorescence levels Fm and Ff (Fig.2). As expected, Fm was little affected by PQex due to their photoreduction during the saturating multiple turnover flash: only oxidised quinones can quench efficiently Chl*a* fluorescence (Amesz and Fork, 1967). However, the small quenching effect of PQex on Ff contrasted with previous results which showed a marked Ff increase following the chemical reduction of the PQ pool (Samson and Bruce, 1996).

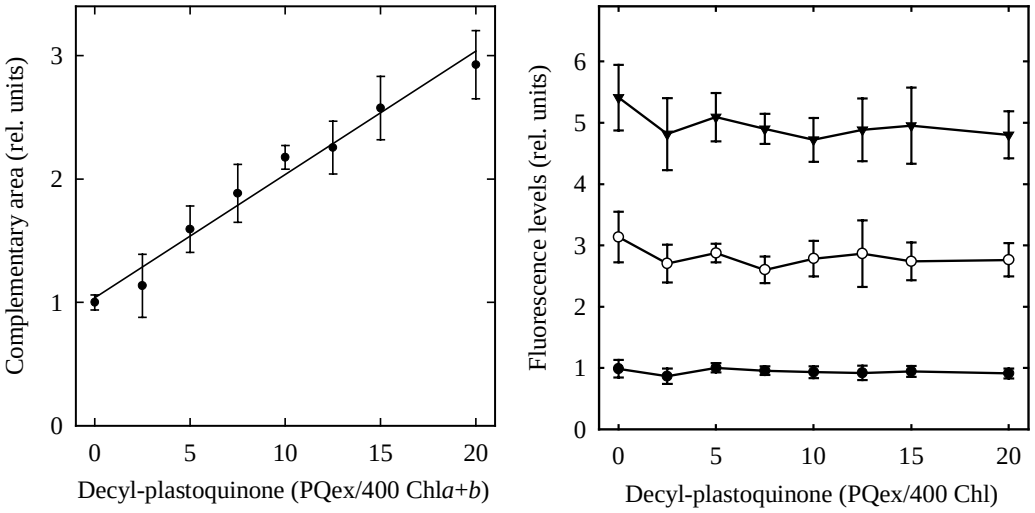

Fig.1 Complementary area above the fluorescence induction curves measured in spinach thylakoids resuspended in presence of different concentrations of decyl-plastoquinone expressed as PQex molecules per 400 Chl*a+b* molecules.
Fig.2 Amplitudes of the fluorescence levels Fo (filled circles), Ff (open circles) and Fm (triangles) in spinach thylakoids resuspended in presence of different concentrations of decyl-plastoquinone (PQex molecules per 400 Chl*a+b* molecules).

Interactive effects between Q_B-site inhibitor and PQex on Chla fluorescence

To determine the relative contributions of free oxidised PQ molecules and the Q_B-bound to the thermal phase, we measured the interactive effects of PQex and DCMU an inhibitor that blocks the binding of oxidised PQ molecules to the Q_B site of PSII, on the fluorescence levels Fo, Ff and Fm. Results shown in Fig.3 indicate that relative to control thylakoids, DCMU markedly increased Fo and Ff but quenched Fm, as previously observed (Crofts and Wraight, 1983; Neubauer and Schreiber, 1987; Vernotte *et al.*, 1979). The amplitude of the variable Chl*a* fluorescence induced by a STF increased by 71% in DCMU-treated thylakoids relative to control thylakoids (Fig.3) whereas the DCMU-induced quenching of Fm accounts for 25% of the thermal phase observed in control thylakoids.

To demonstrate the quenching effect of the oxidised PQ pool on Fm, we measured the effects of PQex on Fm in thylakoids treated or not with DCMU. Compared to the limited effect of PQex on Fm in absence of DCMU (Fig.2), the Fm and Ff quenching caused by PQex increased markedly in DCMU-treated thylakoids (Fig.3). However, when compared to control thylakoids, the PQex quenching effect in presence of DCMU was much more pronounced on the thermal than the photochemical phase of Chl*a* fluorescence. Taken together, the data presented in Figs.2 and 3 could suggest that the thermal phase is more susceptible than the photochemical phase to the non-photochemical quenching effect of the oxidised PQ pool.

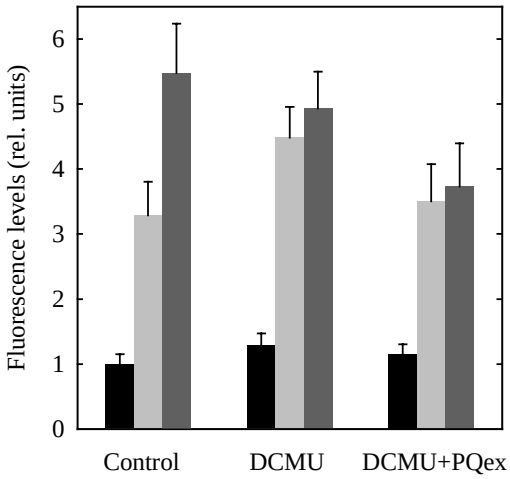

Fig.3 Amplitudes of the fluorescence levels Fo (black), Ff (light grey) and Fm (dark grey) in control thylakoids, in thylakoids resuspended with DCMU (25 μM) or resuspended with DCMU (25 μM) + 10 PQex per 400 Chl*a+b* molecules.

Quenching of the photochemical and thermal phases of Chla fluorescence by 2-methyl-1,4-naphtoquinone

In order to verify the different susceptibilities of the thermal and photochemical phases toward non-photochemical quenching, we measured the fluorescence levels Fo, Ff and Fm in thylakoids resuspended in presence of different concentrations of 2-methyl-1,4-naphtoquinone, an efficient artificial quencher that acts in the chlorophyll antenna. The results showed that the naphtoquinone quenched Fm to a larger extent

than Ff so that at concentrations higher than 40 μM, the thermal phase was practically abolished whereas a significant portion of the photochemical phase (about 50% of the control) remained unquenched (Fig.4). These observations clearly indicate the greater susceptibility of the thermal phase toward non-photochemical quenching. Interestingly, the quenching effect of the naphtoquinone on the photochemical phase is different in intact and DCMU-treated thylakoids. Whereas Ff was less susceptible than Fm to naphtoquinone in thylakoids resuspended in absence of DCMU, Ff and Fm were quenched to a similar extent in DCMU-treated thylakoids (Fig.4). Therefore, DCMU does not solely increase the Ff level but also increases its susceptibility to the non-photochemical quenching effect of 2-methyl-1,4-naphtoquinone.

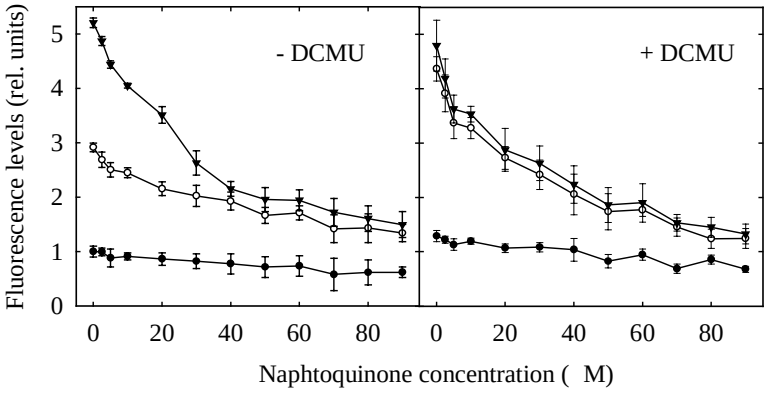

Fig.4 Amplitudes of the fluorescence levels Fo (filled circles), Ff (open circles) and Fm (triangles) in thylakoids resuspended in presence of different concentrations of 2-methyl-1,4-naphtoquinone in absence and in presence of DCMU 25 μM.

Conclusion

The results presented in this study indicate that 1) the Q_B–bound and the free PQ molecules contribute to about 75% and 25% respectively to the thermal phase Chl*a* fluorescence in dark-adapted thylakoids, and 2) the thermal phase of Chl*a* fluorescence is more susceptible to the non-photochemical quenching of oxidised quinones.

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