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In intact leaves, the maximum fluorescence level (F_M) is independent of the redox state of the plastoquinone pool: A DCMU-inhibition study

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Abstract

The effects of DCMU (3-(3′,4′-dichlorophenyl)-1,1-dimethylurea) on the fluorescence induction transient (OJIP) in higher plants were re-investigated. We found that the initial (F_0) and maximum (F_M) fluorescence levels of DCMU-treated leaves do not change relative to controls when the treatment is done in complete darkness and DCMU is allowed to diffuse slowly into the leaves either by submersion or by application via the stem. Simultaneous 820 nm transmission measurements (a measure of electron flow through Photosystem I) showed that in the DCMU-treated samples, the plastoquinone pool remained oxidized during the light pulses whereas in uninhibited leaves, the F_M level coincided with a fully reduced electron transport chain. The identical F_M values with and without DCMU indicate that in intact leaves, the F_M value is independent of the redox state of the plastoquinone pool. We also show that (i) the generally observed F_0 increase is probably due to the presence of (even very weak) light during the DCMU treatment, (ii) vacuum infiltration of leaf discs leads to a drastic decrease of the fluorescence yield, and in DCMU-treated samples, the F_M decreases to the I-level of their control (leaves vacuum infiltrated with 1% ethanol), (iii) and in thylakoid membranes, the addition of DCMU lowers the F_M relative to that of a control sample.

Keywords: Chlorophyll *a* fluorescence; DCMU; OJIP transient; Plastoquinone pool quenching; 820 nm transmission

1. Introduction

DCMU 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea) has been the herbicide of choice in many studies on the induction kinetics of chlorophyll *a* fluorescence. DCMU displaces the secondary quinone acceptor, Q_B, from its binding site at the D1 protein of Photosystem II (PSII) [1,2]. DCMU is not redox active and prevents the re-oxidation of Q_A^- by forward electron transport. Since the fluorescence rise is mainly determined by the redox state of Q_A^- [3], blocking its re-oxidation leads to a considerable simplification of the chlorophyll *a* fluorescence induction (OJIP) kinetics. In leaves, instead of a transient with two intermediate steps (J and I), a single induction phase is observed and the F_M is reached after about 2 ms (=J-step).

The initial (F_0) and the maximum (F_M) values of the fluorescence rise are used to determine the quantum yield of primary photochemistry [$\varphi_{Po}=F_V/F_M=k_P/(k_P+k_N)$, where k_P and k_N are rate constants of photochemistry and other losses of excitation energy, respectively] [4]. The modification of the F_M and F_0 levels in the presence of DCMU would imply a change in the de-excitation rate constants (k_P and k_N).

Abbreviations: ABS/CS, absorption flux per CS; ABS/RC, absorption flux per RC; chl, chlorophyll; CS, cross section; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; ET_0/CS, electron transport flux per CS (at $t=0$); ET_0/RC, electron transport flux per RC (at $t=0$); F_0, initial fluorescence, fluorescence intensity at 20 μs; F_M, maximum fluorescence; J- and I-steps, fluorescence intensities at around 2 and 30 ms, respectively; LED, light-emitting diode; LHCII, light-harvesting chlorophyll *a*/*b* protein complex of PSII; PC, plastocyanin; PQ, plastoquinone; PSI, Photosystem I; PSII, Photosystem II; P680 and P700, reaction center chlorophylls of PSII and PSI, respectively; Q_A and Q_B, primary and secondary quinones of PSII, respectively; RC, reaction center; TR_0/CS, trapped energy flux per CS (at $t=0$); TR_0/RC, trapped energy flux per RC (at $t=0$); ψ_0, probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-; φ_{Po}, maximum quantum yield for primary photochemistry

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Indeed, it has been observed that the addition of DCMU to thylakoid membranes increases the F_0 value and decreases the F_M. The observed increase of F_0 in the presence of DCMU was first explained as a transformation of a part of the active PSII centers into a non-quenching form [5]. Later on, it was shown that in thylakoids, Q_B^- can be found in some of the reaction centers even after a long dark-adaptation [6,7] and that the addition of DCMU leads to a back-transfer of electrons from Q_B^- to Q_A and therefore F_0 increases [8–10]. The decrease of F_M has been attributed to the quenching of fluorescence due to the presence of an oxidized plastoquinone (PQ) pool [11–14]. Since the redox state of the plastoquinone pool changes from oxidized to reduced during a saturating pulse in non-DCMU-inhibited samples, the occurrence of PQ-pool quenching would undermine the basic assumption that the redox state of Q_A is the main factor that determines the fluorescence yield during a saturating pulse (this is used for the quenching analysis method [15,16] and also for the JIP test [17,18]). It has therefore been argued that a correct F_M determination is only possible using a single turnover flash that does not affect the PQ-pool redox status [19,20]. A good overview of the present positions on this issue can be found in two recent publications [21,22].

An alternative interpretation was provided by Vredenberg and Bulychev [23] to explain the lower F_M value of DCMU-treated samples. They proposed that the fluorescence yield is increased by the built-up of a photoelectrochemical field and that the decrease of the F_M to the I-level in the presence of DCMU is due to the absence of the electrochemical field.

We have re-investigated the effects of DCMU on the chlorophyll *a* fluorescence transient of higher plants in different experimental systems (non-detached leaves, leaf discs, vacuum-infiltrated leaf discs and isolated thylakoids) to understand the results published in the literature better. We conclude that in DCMU-treated intact leaves, the same F_0 and F_M values can be obtained as in untreated leaves. The lowering of the F_M level observed in vacuum-infiltrated leaves and isolated thylakoid membranes is suggested to be caused by treatment-induced damage to the thylakoid membranes and/or Photosystem II.

2. Materials and methods

2.1. Plant material

Measurements were carried out on mature leaves of 2- to 4-week-old pea plants (*Pisum sativum* L. cv. Ambassador). Plants were grown in a greenhouse where the temperature was 20–25 °C during the day and about 14 °C at night. Barley (*Hordeum vulgare* L. cv. Triangel) was grown under the same conditions, and for the experiments, leaves of 7-day-old seedlings were used. Other plant species (clover, *Trifolium repens* L., and chickweed, *Stellaria media* L.) were collected in late summer, around the laboratory.

2.2. Thylakoid isolation

Thylakoids were isolated from mature leaves of 2-week-old pea plants. The leaf tissue was homogenized in 40 mM HEPES buffer (pH 7.5), containing 0.4 M sucrose, 1% (w/v) bovine serum albumin (BSA), 5 mM $MgCl_2$, 2 mM Na_2-EDTA (bisodium-ethylenediaminetetraacetic acid) and 15 mM NaCl. The homogenate was filtered through two layers of fine nylon mesh and centrifuged at $3000 \times g$ for 5 min. The plastid pellet was resuspended in the same buffer and was centrifuged at $3000 \times g$ for 5 min. Then, the membrane pellet was resuspended in 40 mM HEPES buffer (pH 7.5) containing 0.4 M sucrose, 5 mM $MgCl_2$ and 15 mM NaCl. The chlorophyll content (measured by the method of Porra et al. [24]) was adjusted to 200 μg ml^{-1}. Thylakoids were kept on ice in darkness and were used within 3 h after isolation.

2.3. DCMU treatment

2.3.1. Submersion of non-detached leaves

Pea plants were put in darkness for about 1 h before the DCMU treatment, and then pairs of leaves were placed into small trays (without detaching them from the plant) filled with 10 ml DCMU solution (if not stated otherwise, the DCMU concentration was 200 μM, and the solution contained 1% ethanol, which was used to dissolve the DCMU). For the controls, leaves were submerged in 1% ethanol or left untreated. The duration of the treatment was about 14 h, and it was carried out in complete darkness. Following the treatment, leaves were removed from the DCMU solution (still not detached and in darkness), wiped and left in the air for about 1 h to avoid possible effects of anaerobiosis. The experimental setup for this type of DCMU treatment is shown in Fig. 1A.

2.3.2. DCMU treatment via the stem

Stems or leaves (5–8 cm long, cut under water) of different plant species were placed in a DCMU solution of 50 μM, for about 30 h, in complete darkness. For control measurements, stems were placed in 0.25% ethanol. The experimental setup is shown in Fig. 1B.

2.3.3. DCMU treatment of leaf discs

Plants were dark-adapted for about 1 h before the treatments. Then, leaf discs of 15-mm diameter were prepared and submerged in a DCMU solution (200 μM, containing 1% ethanol), 1% ethanol or distilled water. Some plants were left untreated. The light intensity during the treatment was ~1 or ~0.3 μmol photons m^{-2} s^{-1}. In

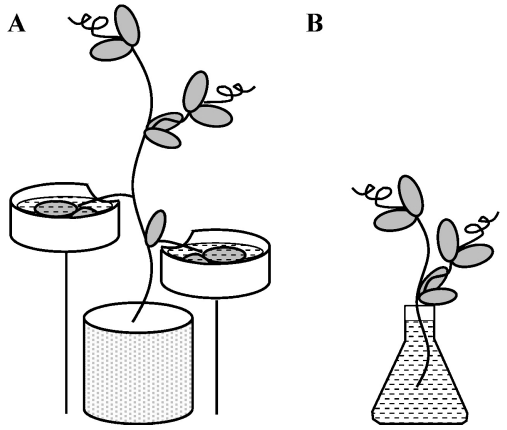

Fig. 1. Experimental setups for DCMU treatments. Panel A: Pairs of pea leaves placed in small trays filled with 10 ml DCMU solution. The duration of the treatment was ~14 h, in complete darkness. Panel B: Stems placed in DCMU solution (50 μM, 0.25% ethanol) for about 30 h, in complete darkness.

addition, a batch of leaf discs was treated in complete darkness. The duration of the treatment was 4 h.

2.3.4. Vacuum infiltration

Leaf discs were vacuum infiltrated with DCMU (80 μM, containing 1% ethanol) or 1% ethanol. Gentle vacuum (−0.8 bar) was provided until air bubbles were formed on the leaf surface, then vacuum was released very rapidly. This was repeated four times and the whole procedure took about 25 min.

2.3.5. DCMU treatment of thylakoids

Immediately before the chlorophyll *a* fluorescence measurements, DCMU was added (10 μM final concentration).

2.4. Measuring equipment

Chlorophyll *a* fluorescence emission was measured with a Handy PEA instrument (Hansatech Instruments, UK). Samples were always dark-adapted for at least 30 min before the measurements and then they were illuminated with continuous light (650 nm peak wavelength, 3000 μmol photons m^{-2} s^{-1} maximum light intensity, if not stated otherwise) provided by an array of three light-emitting diodes focused on a circle of 5 mm diameter of the sample surface. The first reliably measured point of the fluorescence transient is at 20 μs, which was taken as F_0.

820 nm transmission measurements paralleling chlorophyll *a* fluorescence were carried out using a PEA Senior instrument (Hansatech Instruments, UK). The excitation light intensity used was 1800 μmol photons m^{-2} s^{-1}, produced by four LEDs. Far-red light (720 nm peak wavelength, 200 μmol m^{-2} s^{-1} light intensity) and modulated far-red measuring light (820 nm peak wavelength) were provided by two additional LEDs. Further technical details are described in a paper by Schansker et al. [25].

3. Results

3.1. Chlorophyll a fluorescence transients of submerged leaves treated or not with DCMU

In Fig. 2A, the chlorophyll *a* fluorescence transients of untreated leaves and non-detached pea leaves submerged in solutions containing different concentrations of DCMU or 1% ethanol (ethanol was used to dissolve the DCMU) are shown. The figure demonstrates that the J-level increases with increasing DCMU concentration, but the treatments did not change significantly the F_0 (fluorescence intensity at 20 μs) or the F_M values, compared to the 1% ethanol-treated leaves and also to the untreated plant. The F_V/F_M value was about 0.83 for all treatments. At sub-saturating DCMU concentrations (25 and 50 μM DCMU), there was a large variability in the extent of inhibition (data not shown). At the 200 μM DCMU concentration, this variability disappeared and about 95% of the samples were characterized by a single induction phase, similar to the trace presented in Fig. 2A, suggesting full inhibition of PSII. The increase of the DCMU concentration to 400 μM had no additional effects (data not shown). Overnight treatment in 1% ethanol caused a slight increase of the J-level. Further, we note that very similar effects were observed replacing DCMU with the phenolic herbicide *o*-phenanthroline (data not shown).

The analysis of the induction curves measured in the presence of increasing concentrations of DCMU allows us to demonstrate that various JIP test parameters [17,18] behave as predicted on the basis of the model: the specific (per reaction center, RC) and the phenomenological (per excited sample cross section, CS) energy fluxes of the absorbed light (ABS), of the maximum energy trapping (TR_0) and electron transport beyond Q_A^- (ET_0) (inset of Fig. 2A). Practically no changes were found in the presence of DCMU for the fluxes of primary photochemistry (ABS/RC, ABS/CS and TR_0/RC, TR_0/CS). On the other hand, parameters describing electron transport beyond Q_A^- (ET_0/RC and ET_0/CS) gradually decreased with increasing DCMU concentration.

The JIP test allows a separate estimation of the maximum yield of primary photochemistry ($\varphi_{Po}=TR_0/ABS=F_V/F_M$) and for the probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (ψ_0; defined as the flux ratio of ET_0/TR_0). In the inset of Fig. 2A, it can be seen that φ_{Po} is constant for all cases with and without DCMU, while ψ_0 decreases as a function of the DCMU concentration.

In Fig. 2B, the chlorophyll *a* fluorescence transients of DCMU-inhibited (200 μM) and untreated leaves, measured at different light intensities, are presented. The F_0 and F_M values of the DCMU-treated samples were the same as in untreated leaves in the range of 300 to 3000 μmol photons m^{-2} s^{-1}. Light intensities lower than 300 μmol photons m^{-2} s^{-1} were not saturating for control samples, therefore under these conditions, lower F_M values were obtained in

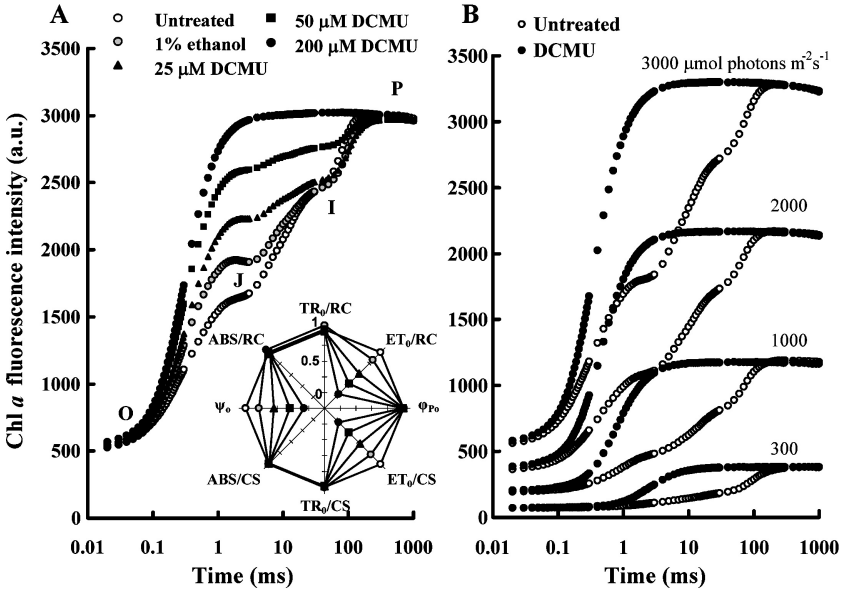

Fig. 2. Panel A: Chlorophyll *a* fluorescence transients of pea leaves treated overnight (according to Fig. 1A) with different concentrations of DCMU (25, 50, and 200 μM in 1% ethanol), 1% ethanol or untreated. Traces are averages of at least 15 repetitions, without any other mathematical treatment. The F_M values of the 200 μM DCMU-treated and the untreated leaves were 3022±129 and 3008±174 (arbitrary units), respectively. The F_0 values were 568±15 (200 μM DCMU) and 544±11 (untreated leaves). In the inset, a multiparametric radar plot of specific fluxes (ABS/RC, TR_0/RC, and ET_0/RC), phenomenological fluxes (ABS/CS, TR_0/CS, and ET_0/CS), the maximum yield of primary photochemistry ($\varphi_{Po}=TR_0/ABS=F_V/F_M$) and for the probability (at time 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^- (ψ_0; defined as the flux ratio of ET_0/TR_0) are presented according to the equations of the JIP test (Strasser et al. 2004 [18]). All values are expressed relative to the values of the untreated sample. The light absorbed per leaf area cross section (ABS/CS) was considered to be proportional to F_M. Panel B: Chlorophyll *a* fluorescence transients of DCMU-treated (200 μM, 1% ethanol, treated according to Fig. 1A) and untreated leaves measured at different light intensities.

control samples than in DCMU-treated samples (data not shown).

3.2. Simultaneous 820 nm transmission and chlorophyll a fluorescence measurements

In order to check if the 200 μM DCMU treatment used above completely blocked the electron flow from PSII, 820 nm transmission measurements were made simultaneously with chlorophyll *a* fluorescence measurements. Transmission changes at 820 nm reflect the redox states of P700 and plastocyanin (PC) [26,27] and were used here to monitor electron flow through Photosystem I (PSI). As shown in the left panel of Fig. 3, in control leaves (leaves treated with 1% ethanol or left untreated), a red light pulse (1800 μmol photons m^{-2} s^{-1}, 1.5 s) induced an initial oxidation of PC and P700 (seen as a decrease of the transmission level) followed by re-reduction as a consequence of the arrival of electrons from PSII and a reduction of the acceptor side of PSI [25]. Subsequently, a far-red pulse (200 μmol photons m^{-2} s^{-1}, 6 s) was given in order to oxidize the electron transport chain (Fig. 3, middle panel). During the far-red

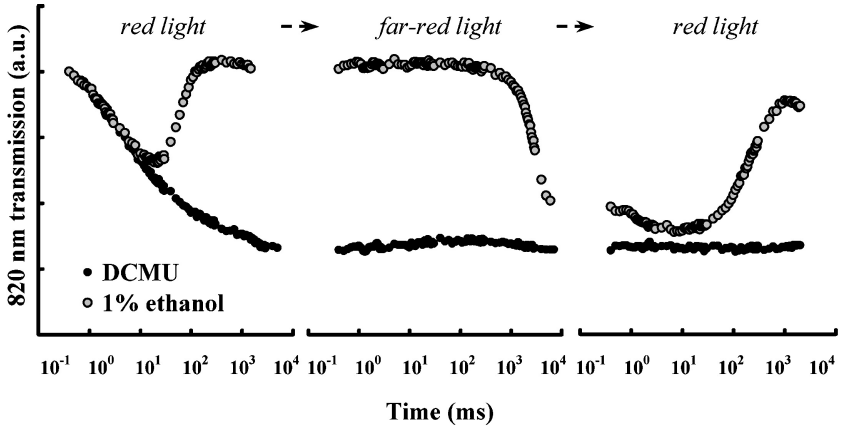

Fig. 3. 820 nm transmission signals of DCMU-treated (200 μM, 1% ethanol, treated according to Fig. 1A) and 1% ethanol-treated leaves. Signals were recorded during red light illumination (1800 μmol photons m^{-2} s^{-1}, 1.5 s for control and 5 s for DCMU-treated leaves) followed by far-red light illumination (6 s, 200 μmol photons m^{-2} s^{-1}) and red light illumination (2 s, 1800 μmol photons m^{-2} s^{-1}). There was about 250 μs of darkness between the light pulses.

illumination, P700 and PC were kept initially in the reduced state by electrons arriving from the reduced PQ-pool. The oxidation of the PQ-pool was followed by the oxidation of PC and P700, observed as a decrease of the 820 nm transmission level. During the final red pulse (Fig. 3, right panel), a further decrease is observed. The far-red pulse was too short for a full oxidation of all the P700 and PC and the much stronger red pulse (the red light is about 36-fold more effective in PSI excitation than the far-red light used; G. Schansker, unpublished observation) completed the oxidation process. Following this phase, the 820 nm transmission level increases, which shows that the electron transport chain, including PC and P700, becomes reduced again.

In the case of DCMU-treated samples (200 μM), the 820 nm transmission continuously decreased during the first red-light pulse until a steady-state level was established (at about 3 s). The duration of this process may seem rather long, but the pool of stromal electrons has to be emptied before the minimum transmission level can be reached (see Ref. [25] for a demonstration of this process). During the subsequent far-red and red light pulses, no further 820 nm transmission changes were observed, indicating that PC and P700 were oxidized and remained oxidized. A (at least partial) reduction of the PQ-pool would have resulted in a transient increase of the 820 nm signal during the far-red pulse, as we observed in the presence of methylviologen [28]. This means that no electrons were passing the DCMU block and therefore the PQ-pool remained in the oxidized state during the different light pulses. For the fluorescence induction measurements in the presence of DCMU, this implies that at the F_M level, the PQ-pool was in the oxidized state.

3.3. F_0 and F_M values in leaves of different plant species treated by DCMU via the stem

In Fig. 4, the F_0 and F_M values of leaves of four different plants species (pea, chickweed, barley and clover) to which a DCMU solution or 0.25% ethanol was applied via the stem (according to Fig. 1B) are shown. In this case, too, the F_0 and F_M values were practically the same for DCMU-treated and control samples. This experiment shows that the unchanged F_0 and F_M values are not only a peculiarity of the pea plants or the treatment depicted in Fig. 1A, but a more general phenomenon.

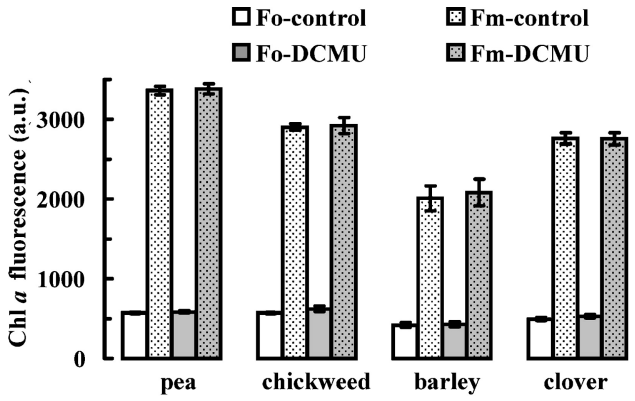

Fig. 4. F_0 and F_M values of leaves of different plant species to which a 50 μM DCMU solution or 0.25% ethanol was applied via the stem (according to Fig. 1B). The standard deviation is indicated, $n \geq 15$.

3.4. Possible factors leading to the usually observed F_0 increase and F_M decrease

Fig. 5A shows chlorophyll *a* fluorescence transients of non-detached leaves and leaf discs submerged in distilled water or in DCMU in the presence of different light intensities. Leaves or leaf discs were dark-adapted for 30 min after the different treatments, and then chlorophyll *a* fluorescence was measured. The figure shows that the submersion of leaf discs in distilled water for 4 h caused a few percent (4–7%) decrease in the overall fluorescence yield compared to the untreated plant. 1% ethanol had no additional effect on leaf discs in this 4-h time range (data not shown). We also note that the F_M values of DCMU-treated leaves were the same as those of its controls (leaf discs treated in distilled water or in 1% ethanol).

Fig. 5A also shows that when the DCMU treatment was carried out in the presence of low light, the F_0 significantly increased (at 1 μmol photons m^{-2} s^{-1} light intensity, the F_0 was about 48.5% higher, and at 0.3 μmol photons m^{-2} s^{-1}, it was about 39% higher than the F_0 of the non-DCMU-treated leaf discs). When the treatment was done in complete darkness, the F_0 value still increased by about 11%. This could be avoided by using plants that were dark-adapted overnight before the start of the DCMU treatments (data not shown).

Vacuum infiltration of leaf discs resulted in a strong (about 45%) decrease in the overall fluorescence yield (Fig. 5B). In addition, the F_M value of DCMU-treated leaf discs decreased to the I-step of the leaf discs that were vacuum-infiltrated with 1% ethanol (see the lower two curves in Fig. 3B). Similar results were obtained by Kramer et al. [29]. The F_0 value increased by about 20%, which was due to the use of dim light during vacuum infiltration.

Measurements carried out on thylakoids showed similar effects (Fig. 5C). By the addition of DCMU, the F_M decreased by about 10%, and the F_0 increased by 17% compared to control thylakoids. Thus, the results of e.g. Vernotte et al. [11] were confirmed. However, in intact leaves, the situation seemed to be different.

4. Discussion

4.1. Treatment conditions

Overnight submersion of non-detached leaves in DCMU solution in complete darkness was used to obtain inhibited leaves that have F_0 and F_M values identical to those of

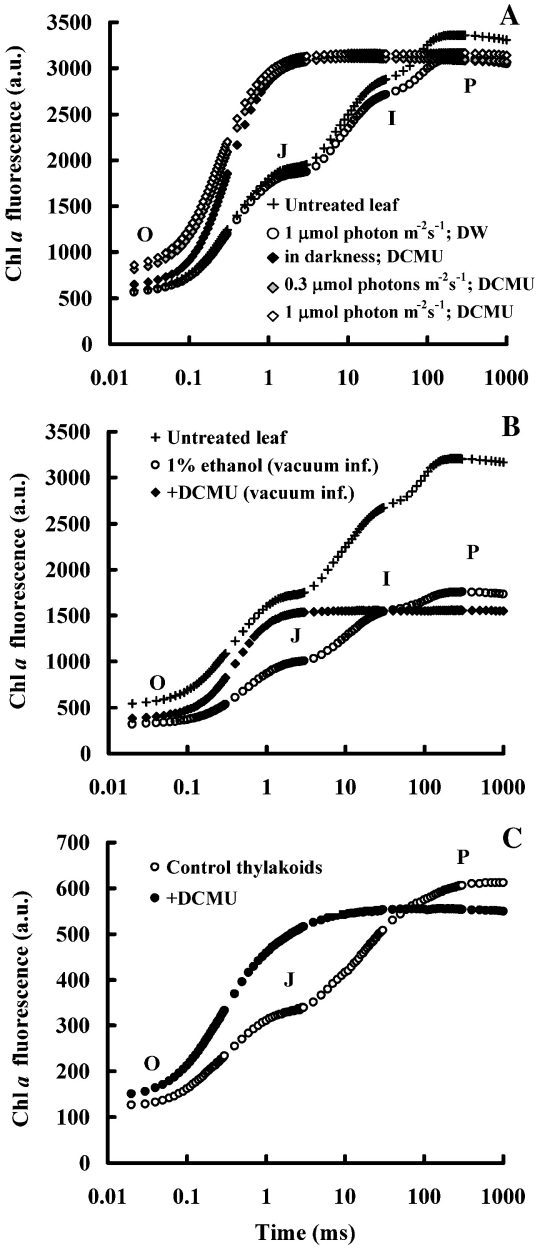

Fig. 5. Panel A: Chlorophyll *a* fluorescence induction transients of untreated pea leaves, leaf discs treated with DCMU (200 μM in 1% ethanol) under different light intensities and leaf discs treated with distilled water (DW); $n \geq 15$. Panel B: Chlorophyll *a* fluorescence induction transients of untreated pea leaves, vacuum-infiltrated leaf discs with DCMU or 1% ethanol; $n \geq 15$. Panel C: Pea thylakoids with or without DCMU; $n = 3$.

untreated leaves (Fig. 2A and B). It is important to note that the long dark-incubation time was only necessary to allow a full dark-adaptation of the F_0 level. As described in the Results section, a full inhibition of PSII is achieved within 4 h and also under those circumstances, there is no effect on the F_M level compared to its controls (leaf discs treated in distilled water or 1% ethanol). We also note that the OJIP-fluorescence transients of uninhibited samples measured after 1 h or 14 h dark-adaptation are very much alike (data not shown). Plants are subjected to long dark periods during their natural life cycle (every night). Therefore, we can assume that the long dark-adaptation did not cause an inhibition of the photosynthetic apparatus.

Unchanged F_0 and F_M values were observed also when leaves were not submerged, but the DCMU was applied via the stem (Fig. 1B). This is a strong indication that possible submersion-related artifacts did not play a role under our experimental conditions.

Anaerobiosis is associated with a reduction of the PQ-pool [30]. After the treatment by submersion (Fig. 1A), leaves were incubated in the air for at least 1 h before chlorophyll *a* fluorescence measurements in order to avoid possible effects of anaerobiosis. One hour is thought to be sufficient for the re-oxidation of the PQ-pool [31]. We have two experimental observations demonstrating that the PQ-pool was not reduced at the beginning of the measurements: (1) The reduction of the PQ-pool leads to a strong increase of the J-level [31] but this was not observed in the case of the control samples (leaves submerged in 1% ethanol). (2) A (partially) reduced PQ-pool would delay the initial 820 nm transmission decrease since the PQ molecules have to be oxidized before $P700^+$ and PC^+ can accumulate. Such a delay was not observed (see also [25]).

4.2. The effect of DCMU on the F_0 value

It is a commonly held view that the addition of DCMU results in an increase of the F_0 value. In this study, we show that this effect can be avoided by long dark adaptation of non-detached leaves before and during DCMU treatments.

4.3. The effect of DCMU on the F_M value

The interaction between quinones and excited chlorophylls results in energy dissipation without electron transfer [32]. Several studies have demonstrated that in isolated thylakoids, exogenously added quinones in the oxidized form can dissipate energy by interacting with either light-harvesting complexes of PSII or chlorophylls at or near PSII reaction centers [33–35]. It has also been shown that in thylakoids, the presence of an oxidized PQ-pool leads to quenching of the F_M value [11–14]. This result is also confirmed by our data (Fig. 5C). In PSII membrane fragments subjected to a detergent treatment (e.g. Triton X-100), the effect of the oxidation state of the PQ-pool on the F_M value is even more pronounced than in thylakoids [36–38]. These data can be explained by assuming that damage caused to PSII complexes by each additional treatment progressively increases the accessibility of oxidized PQ molecules to excited chlorophyll molecules.

Vacuum infiltration is thought to cause an osmotic shock and it may also cause mechanical modification of thylakoid membranes, just as thylakoid isolation proce-

dures and detergent treatments. This may explain why we observe a significant decrease of the F_M value in DCMU-treated samples in the case of vacuum-infiltrated leaves (Fig. 5B). Similar results were obtained also by Kramer et al. [29].

In intact leaves, the F_M values are the same in the presence or absence of DCMU (Fig. 2). In the case of control leaves, the PQ-pool is in its reduced state at the F_M level, whereas in the case of DCMU-treated leaves, the PQ-pool is oxidized (see Fig. 3). This means that in the case of intact leaves, the F_M value is not influenced by the redox state of the PQ-pool. It may indicate that in intact thylakoid membranes, the excited antenna molecules are not accessible to oxidized PQ molecules. This is supported by the observations of Kurreck et al. [37]. These authors suggested on the basis of 90 K fluorescence spectra and binding studies with radioactively labeled PQ-9 (performed by Satoh et al. [39]) that in PSII membrane fragments, PQ molecules interact with the chlorophylls bound to CP47 (a unit of the inner antenna) and not with the more accessible outer antenna.

Literature data show that it is possible to prepare intact chloroplasts that have identical F_M (and also F_0) values in the presence and absence of DCMU [40]. On the basis of these data and our results, we think that the extent of PQ-pool quenching could provide a tool to assess the intactness of the PSII–protein complex and the quality of the isolation procedure.

The unchanged F_M levels in leaves in the presence of DCMU indicate that the hypothesis that the IP-phase of the chlorophyll *a* induction transient corresponds to the removal of PQ-pool quenching (see e.g. Refs. [12,41]) cannot be correct. Vredenberg and Bulychev [23] suggested that the difference in the F_M level with and without DCMU is caused by a limited built-up of the photo-electrochemical field. The nearly identical F_M levels observed in this study in leaves contradict this suggestion.

We have shown that the submersion of leaf discs in water or in a DCMU solution decreases the overall fluorescence yield by a few percent (Fig. 5A). This observation emphasizes the importance of the preparation of appropriate controls (leaf discs submerged in water or 1% ethanol) and not to compare DCMU-treated, submerged leaf discs to untreated, non-detached leaves.

Finally, our data suggest that the best DCMU treatment is obtained through a passive (slow) diffusion of DCMU into non-detached leaves that are kept in complete darkness (as shown in Fig. 1A and B). In this way, increases of the F_0 and decreases of the F_M values are avoided.

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References

[1] B.R. Velthuys, Electron-dependent competition between plastoquinone and inhibitors for binding to Photosystem II, FEBS Lett. 126 (1981) 277–281.
[2] C.A. Wraight, Oxidation-reduction physical chemistry of the acceptor quinone complex in bacterial photosynthetic reaction centers: evidence for a new model of herbicide activity, Isr. J. Chem. 21 (1981) 348–354.
[3] L.N.M. Duysens, H.E. Sweers, Mechanisms of two photochemical reactions in algae as studied by means of fluorescence, Studies on Microalgae and Photosynthetic Bacteria, Special Issue of Plant and Cell Physiology, Japanese Society of Plant Physiologists, University of Tokyo Press, Tokyo, Japan, 1963, pp. 353–372.
[4] M. Kitajima, W.L. Butler, Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone, Biochim. Biophys. Acta 376 (1975) 105–115.
[5] P. Bennoun, Y.-S. Li, New results on the mode of action of 3-(3,4-dichlorophenyl)-1,1-dimethylurea in spinach chloroplasts, Biochim. Biophys. Acta 292 (1973) 162–168.
[6] H. Laasch, W. Urbach, U. Schreiber, Binary flash-induced oscillations of [^{14}C]DCMU binding to the Photosystem II acceptor complex, FEBS Lett. 159 (1983) 275–279.
[7] A.W. Rutherford, Govindjee, Y. Inoue, Charge accumulation and photochemistry in leaves studied by thermoluminescence and delayed light emission, Proc. Natl. Acad. Sci. U. S. A. 81 (1984) 1107–1111.
[8] B.R. Velthuys, J. Amesz, Charge accumulation at the reducing side of system 2 of photosynthesis, Biochim. Biophys. Acta 333 (1974) 85–94.
[9] A.W. Rutherford, A.R. Crofts, Y. Inoue, Thermoluminescence as a probe of Photosystem II photochemistry. The origin of the flash-induced glow peaks, Biochim. Biophys. Acta 682 (1982) 457–465.
[10] H. van Gorkom, Fluorescence measurements in the study of Photosystem II electron transport, in: Govindjee, J. Amesz, D.C. Fork (Eds.), Light Emission by Plants and Bacteria, Academic Press, New York, 1986, pp. 267–289.
[11] C. Vernotte, A.L. Etienne, J.-M. Briantais, Quenching of the system II chlorophyll fluorescence by the plastoquinone pool, Biochim. Biophys. Acta 545 (1979) 519–527.
[12] C. Neubauer, U. Schreiber, The polyphasic rise of chlorophyll fluorescence upon onset of strong continuous illumination: I. Saturation characteristics and partial control by the Photosystem II acceptor side, Z. Naturforsch. 42c (1987) 1246–1254.
[13] B.-D. Hsu, J.-Y. Lee, Fluorescence quenching by plastoquinone in an oxygen-evolving Photosystem-II-enriched preparation, J. Photochem. Photobiol. 30 (1995) 57–61.
[14] P. Haldimann, M. Tsimilli-Michael, Non-photochemical quenching of chlorophyll a fluorescence by oxidised plastoquinone: new evidences based on modulation of the redox state of the endogenous plastoquinone pool in broken spinach chloroplasts, Biochim. Biophys. Acta 1706 (2005) 239–249.
[15] U. Schreiber, U. Schliwa, W. Bilger, Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer, Photosynth. Res. 10 (1986) 51–62.
[16] O. van Kooten, J.F.H. Snel, The use of chlorophyll fluorescence nomenclature in plant stress physiology, Photosynth. Res. 25 (1990) 147–150.
[17] B.J. Strasser, R.J. Strasser, Measuring fast fluorescence transients to address environmental questions: the JIP test, in: P. Mathis (Ed.),

Photosynthesis: From Light to Biosphere, vol. V, Kluwer Academic Publishers, Dordrecht, 1995, pp. 977–980.
[18] R.J. Strasser, A. Srivastava, M. Tsimilli-Michael, Analysis of the chlorophyll *a* fluorescence transient, in: G. Papageorgiou, Govindjee (Eds.), Chlorophyll Fluorescence: A Signature of Photosynthesis, Advances in Photosynthesis and Respiration, vol. 19, Kluwer Academic Publishers, The Netherlands, 2004, pp. 321–362.
[19] P.G. Falkowski, Z. Kolber, D. Mauzerall, A comment on the call to throw away your fluorescence induction apparatus, Biophys. J. 66 (1994) 923–928.
[20] O. Prasil, Z. Kolber, J.A. Berry, P.G. Falkowski, Cyclic electron flow around Photosystem II in vivo, Photosynth. Res. 48 (1996) 395–410.
[21] U. Schreiber, Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview, in: G. Papageorgiou, Govindjee (Eds.), Chlorophyll Fluorescence: A Signature of Photosynthesis, Advances in Photosynthesis and Respiration, vol. 19, Kluwer Academic Publishers, The Netherlands, 2004, pp. 279–319.
[22] P.G. Falkowski, M. Koblížek, M. Gorbunov, Z. Kolber, Development and application of variable chlorophyll fluorescence techniques in marine ecosystems, in: G. Papageorgiou, Govindjee (Eds.), Chlorophyll Fluorescence: A Signature of Photosynthesis, Advances in Photosynthesis and Respiration, vol. 19, Kluwer Academic Publishers, The Netherlands, 2004, pp. 757–778.
[23] W.J. Vredenberg, A. Bulychev, Photo-electrochemical control of Photosystem II chlorophyll fluorescence in vivo, Bioelectrochemistry 57 (2002) 123–128.
[24] R.J. Porra, W.A. Thompson, P.E. Kriedeman, Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy, Biochim. Biophys. Acta 975 (1989) 384–394.
[25] G. Schansker, A. Srivastava, Govindjee, R.J. Strasser, Characterization of the 820-nm transmission signal paralleling the chlorophyll a fluorescence rise (OJIP) in pea leaves, Funct. Plant Biol. 30 (2003) 785–796.
[26] J. Harbinson, C.L. Hedley, The kinetics of P-700^{+} reduction in leaves: a novel in situ probe for thylakoid functioning, Plant Cell Environ. 12 (1989) 357–369.
[27] C. Klughammer, U. Schreiber, Analysis of light-induced absorbance changes in the near-infrared spectral region: I. Characterization of various components in isolated chloroplasts, Z. Naturforsch. 46c (1991) 233–244.
[28] G. Schansker, S.Z. Tóth, R.J. Strasser, Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of Photosystem I in the Chl a fluorescence rise OJIP, Biochim. Biophys. Acta 1706 (2005) 250–261.
[29] D.M. Kramer, G. DiMarco, F. Loreto, Contribution of plastoquinone quenching to saturation pulse-induced rise of chlorophyll fluorescence in leaves, in: P. Mathis (Ed.), Photosynthesis: From Light to Biosphere, vol. I, Kluwer Academic Publishers, Dordrecht, 1995, pp. 147–150.
[30] G.C. Harris, U. Heber, Effects of anaerobiosis on chlorophyll fluorescence yield in spinach (*Spinacia oleracea*) leaf discs, Plant Physiol. 101 (1993) 1169–1173.
[31] P. Haldimann, R.J. Strasser, Effects of anaerobiosis as probed by the polyphasic chlorophyll a fluorescence rise kinetic in pea (*Pisum sativum* L.), Photosynth. Res. 62 (1999) 67–83.
[32] D. Huppert, P.M. Rentzepis, G. Tollin, Picosecond kinetics of chlorophyll and chlorophyll/quinone solutions in ethanol, Biochim. Biophys. Acta 440 (1976) 356–364.
[33] J. Amesz, D.C. Fork, Quenching of chlorophyll fluorescence by quinones in algae and chloroplasts, Biochim. Biophys. Acta 143 (1967) 97–107.
[34] K. Pfister, H.K. Lichtenthaler, G. Burger, H. Musso, M. Zahn, The inhibition of photosynthetic light reaction by halogenated napthoquinones, Z. Naturforsch. 36c (1981) 645–655.
[35] S.J. Berens, J. Scheele, W.L. Butler, D. Magde, Time-resolved fluorescence studies of spinach chloroplasts—evidence for the heterogeneous bipartite model, Photochem. Photobiol. 42 (1985) 51–57.
[36] E. Lam, B. Baltimore, W. Ortiz, S. Chollar, A. Melis, R. Malkin, Characterization of a resolved oxygen-evolving Photosystem II preparation from spinach thylakoids, Biochim. Biophys. Acta 724 (1983) 201–211.
[37] J. Kurreck, R. Schödel, G. Renger, Investigation of the plastoquinone pool size and fluorescence quenching in thylakoid membranes and Photosystem II (PSII) membrane fragments, Photosynth. Res. 63 (2000) 171–182.
[38] P. Pospíšil, H. Dau, Chlorophyll fluorescence transients of Photosystem II membrane particles as a tool for studying photosynthetic oxygen evolution, Photosynth. Res. 65 (2000) 41–52.
[39] K. Satoh, S. Katoh, R. Dostatni, W. Oettmeier, Herbicide and plastoquinone-binding proteins of Photosystem II reaction center complexes from the thermophilic cyanobacterium, *Synechococcus* sp., Biochim. Biophys. Acta 851 (1986) 202–208.
[40] U. Schreiber, A. Krieger, Two fundamentally different types of variable chlorophyll fluorescence in vivo, FEBS Lett. 397 (1996) 131–135.
[41] D. Lazár, M. Brokeš, J. Nauš, L. Dvořák, Mathematical modelling of 3-(3′,4′-dichlorophenyl)-1,1′-dimethylurea action in plant leaves, J. Theor. Biol. 191 (1998) 79–86.