ELSEVIER

Journal of Photochemistry and Photobiology B: Biology 31 (1995) 163–169

Journal of PHOTOCHEMISTRY AND PHOTOBIOLOGY B:BIOLOGY

Differential effects of dimethylbenzoquinone and dichlorobenzoquinone on chlorophyll fluorescence transient in spinach thylakoids

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Received 5 April 1995; accepted 22 May 1995

Abstract

In isolated thylakoids, the differential effects of 2,5-dimethyl-*p*-benzoquinone (DMQ) and 2,6-dichloro-*p*-benzoquinone (DCBQ) on chlorophyll *a* (Chl *a*) fluorescence transients and O_2 evolution data have been used to differentiate between active and inactive photosystem II (PSII) centers. This conclusion was challenged by Lavergne and Leci (*Photosynth. Res., 35* (1993) 323–343). Thus we have systematically re-investigated this phenomenon using different concentrations of DMQ and DCBQ in thylakoids exposed to various light intensities. We show that the differential effects of DMQ and DCBQ on Chl *a* fluorescence transients in spinach thylakoids include a larger decrease in the variable Chl *a* fluorescence by DCBQ than by DMQ and a decrease in the F_o level with increasing [DCBQ] but not [DMQ] in the 5–150 μM range. These differential effects confirm the results of Lavergne and Leci, and thus sustain the conclusion that the effects of DMQ and DCBQ on Chl *a* fluorescence cannot be used to distinguish between active and inactive PSII centers. The data show that the decrease in Chl *a* fluorescence follows Stern–Volmer behavior. Furthermore, our data confirm that the rate of O_2 evolution is higher in the DCBQ-supported Hill reaction than in the DMQ- supported Hill reaction. These results can be explained by a more efficient ability of DCBQ to oxidize the plastoquinol pool or a more efficient ability of DCBQ to displace Q_B (secondary bound plastoquinone, a two-electron acceptor of PSII), rather than activating the inactive PSII centers. However, our results do not challenge the existence of inactive PSII.

Keywords: Chlorophyll *a* fluorescence; Active/inactive photosystem II; Dichlorobenzoquinone; Dimethylbenzoquinone

1. Introduction

In normally functioning photosystem II (PSII) complexes, electrons originating in water molecules reduce bound plastoquinones (PQs) [1]. A two-electron reduction of a PQ, labeled as Q_B, requires two sequential photochemical reactions involving the participation of a reaction center (RC) chlorophyll (Chl) of PSII, P680, a pheophytin and a one-electron acceptor bound PQ Q_A. The double reduction of Q_B is followed by protonation and release of plastoquinol (PQH_2) into the thylakoid membrane [2–4].

Although the above view of PSII is sufficient to account for an extensive number of observations, there are many experiments that demonstrate the heterogeneous behavior of PSII (see, for example, Refs. [5] and [6]). Major support for the existence of PSII heterogeneity comes from the interpretation of Chl *a* fluorescence measurements. Chl *a* fluorescence changes as a function of the time of continuous illumination (fluorescence induction or transient) are, in general, understood as the filling up of the PQ pool [7–9]. Melis and Homann [10] have observed that, in 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)-treated chloroplasts, the area over the Chl *a* fluorescence induction curve increases with biphasic first-order kinetics. Assuming the existence of a heterogeneous pool of photochemical centers, Melis and Homann [11] analyzed the growth of the area over the fluorescence curve, and labeled the fast PSII centers as α and the relatively slower PSII centers as β. Lavergne [12,13] investigated the Q_B site of PSII by Chl *a* fluorescence measurements and concluded that there are two types of PSII centers, one with Q_B and one without Q_B, the non-Q_B type. The non-Q_B centers are impaired at the Q_B site and do not transfer electrons efficiently to the PQ pool. These have often been considered as equivalent to the "inactive" (or slow) PSII centers of other investigators [14–16]. Melis [17] proposed that the inactive centers are located in the stromal part of the thylakoid membrane and have smaller light-harvesting

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1011-1344/95/$09.50
SSDI 1011-1344(95)07177-6

antennae size, whereas the active centers are located in the granal region and have larger light- harvesting antennae size.

A number of experimental approaches have been used to quantitate the number of active and inactive PSII centers. One method for the measurement of centers inactive in PQ reduction is based on the amplitude of the electrochromic band shift at 518 nm (ΔA_{518}), due to photochemical charge separation in RCs in spinach, studied under both in vitro and in vivo conditions [18,19]. These and other results establish the existence of inactive PSIIs in the range of 10%–30% [6].

A different method is based on the inability of the inactive centers to transfer electrons from Q_A^- to Q_B. Hence it has been suggested that, on low light illumination, the photochemical activity can be revealed by a fluorescence yield increase from F_o to the intermediate plateau F_{pl}, which further increases due to the electron flow to the PQ pool in active PSII centers, reaching the maximum level F_m [20]. In this method, the relative Chl *a* fluorescence yield of the transition from F_o to F_{pl} provides a measure of the relative concentration of the PSII Q_B-non-reducing inactive centers. On this basis, Guenther et al. [20] quantitated the PSII Q_B-non-reducing inactive centers in *Dunaliella salina* cells, and suggested that approximately 25% of all PSII centers in this green algae are inactive.

Graan and Ort [15] (cf. Ref. [21]) suggested that "inactive" centers (about 40%) could be reactivated by providing them with an appropriate exogenous acceptor, such as 2,6-dichloro-*p*-benzoquinone (DCBQ) or other halogenated quinones. The methyl-substituted benzoquinones (e.g. 2,5-dimethyl-*p*-benzoquinone (DMQ)) are much less effective in interacting with these centers, explaining the higher rates of Hill reaction with DCBQ than with DMQ. Based on the differential effects of DCBQ and DMQ on Chl *a* fluorescence transients, Cao and Govindjee [22] concluded that the OID phase of the Chl *a* fluorescence induction curve reflects the reduction of the electron acceptor Q_A to Q_A^- in the "inactive" (or slow) PSII centers since they observed that DCBQ, not DMQ, abolishes this OID phase. Lavergne and Leci [23] examined further the effects of DMQ and DCBQ, and concluded that the electron acceptor DCBQ cannot restore electron transfer in the inactive centers, in disagreement with previous conclusions [15,21,22]. Lavergne and Leci [23] observed a decrease in the "O" level by DCBQ and thus challenged the premise of the conclusion by Cao and Govindjee [22] since they had observed no such quenching. In view of the differences in the experimental results between Cao and Govindjee [22] and Lavergne and Leci [23], we have re-examined the effects of different concentrations of DMQ and DCBQ on Chl *a* fluorescence transients and O_2 evolution. We confirm the results of Lavergne and Leci [23] that DCBQ not only decreases the variable Chl *a* fluorescence, but also F_o. The results of Cao and Govindjee [22], using a camera-shutter instrument, can be explained only if an error was made in marking F_o of their DMQ sample to which DCBQ was added.

2. Materials and methods

Chloroplast thylakoid membranes were isolated from market spinach leaves by homogenization in a Waring blender for 10 s in a grinding buffer containing 50 mM Tricine, 300 mM NaCl and 3 mM $MgCl_2$ (pH 7.5). The slurry was filtered through two layers of nylon (36 μm) plus two layers of muslin cloth, and centrifuged for 5 min at 4000 rev min^{-1} (Universal/K2S, Hettich, Tuttlingen, Germany). The pellet was washed once with a resuspension buffer containing 200 mM sorbitol, 20 mM Tricine and 5 mM NaCl (pH 7.5) and finally resuspended in the same buffer at a Chl concentration of 2.0–2.5 mg ml^{-1}. Aliquots (1 ml) were quickly frozen and stored in liquid nitrogen (77 K). Just prior to the experiments, thylakoids were thawed and diluted to 45 μg ml^{-1} of Chl in a buffer containing 300 mM sucrose, 25 mM 2-(*n*-morpholino)ethanesulfonic acid (MES), 10 mM NaCl and

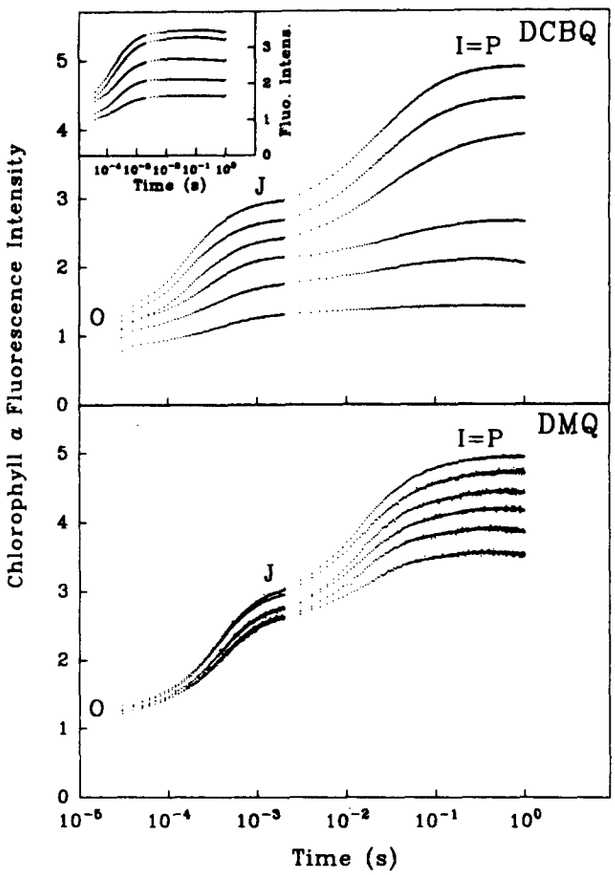

Fig. 1. Effect of different concentrations of DCBQ (top panel) and DMQ (bottom panel) on Chl *a* fluorescence transients of spinach thylakoids at room temperature. The thylakoids were suspended in the reaction medium containing 0.3 M sucrose, 25 mM MES buffer (pH 6.5), 10 mM NaCl and 5 mM $MgCl_2$. From top to bottom, the transients were obtained with either 0, 5, 20, 50, 80 and 160 μM of DCBQ or 0, 5, 20, 40, 80 and 160 μM of DMQ. Samples were dark adapted for 5 min before exposure to 650 nm light of 600 W m^{-2} intensity. The inset shows the effect of 0, 20, 40, 80 and 160 μM of DCBQ on Chl *a* fluorescence transients in the presence of 20 μM DCMU. (For a discussion of the nomenclature of the transient points, see Ref. [27].)

5 mM $MgCl_2$ (pH 6.5). The process of freezing and thawing disrupts the chloroplast envelope membranes and provides a suspension of thylakoid membranes, not intact chloroplasts. Experiments performed with freshly prepared spinach thylakoids gave similar results.

The Chl concentration of the thylakoid membrane was determined according to the method of Porra et al. [24]. All the experiments were performed with recrystallized DCBQ and DMQ. No differences in the results were found when reagent grade DCBQ and DMQ from Eastman Kodak were used, or samples donated by Dr. Jerome Lavergne and Professor Donald Ort.

Chl *a* fluorescence transients were measured by a plant efficiency analyzer (PEA; Hansatech Ltd., King's Lynn, Norfolk, UK) using various light intensities. Light was provided by an array of six light-emitting diodes (peak, 650 nm), focused on the sample surface to provide homogeneous illumination over the exposed area of the sample (diameter, 4 mm).

Chl *a* fluorescence signals were detected using a PIN photocell after passing through a long pass filter (50% transmission at 720 nm). All the experiments were performed with 500 μl aliquots of thylakoid suspension (50 μg ml^{-1}) in 1 cm diameter vials. The optical thickness of the sample was 5 mm, and the diameter of the sample area irradiated was 4 mm. Thylakoid samples were dark-adapted for 5 min, and then in darkness, DCBQ or DMQ was added followed by vortexing of the samples also in darkness, before fluorescence measurements began. Chl *a* fluorescence signals were recorded in a time span from 10 μs to 2 min with a data acquisition rate of 10 μs for the first 2 ms and 12 bit resolution. The fluorescence signal at 40 μs has been considered as F_o since only data points after 40 μs were reliable and free from the artifacts of the electronics of the instrument. Extrapolated F_o values from the kinetics of the fluorescence rise match this value within the error limits ($\pm 3\%$). Although the data were plotted up to 1 s, F_m was obtained at long times when the electron acceptors were exhausted. Results are plotted on logarithmic as well as linear time scales.

The half-fluorescence rise times in the presence of 20 μM DCMU at 12, 60, 300 and 600 W m^{-2} light intensities were 20, 5, 1 and 0.3 ms respectively in our experiments. Although Lavergne and Leci [23] used lower light intensities, giving them a half-fluorescence rise time of 50–100 ms in the presence of DCMU, our results are comparable with theirs.

For the O_2 evolution measurements, thylakoid suspensions were incubated for 5 min in the dark with different concentrations of quinones in DW-1 Hansatech cuvettes. Samples were illuminated with 300 W m^{-2} of red actinic light from a Schott lamp (Schott Electronics KL1500, with red filter RG 610). Rates of O_2 evolution were measured during the first minute after illumination.

3. Results and discussion

The effects of quenching of different quinones on F_o and F_m have been documented previously at both room and low temperatures [25,26]. It is imperative that we distinguish between the quenching of F_o, which is due to quenching of antenna fluorescence, and quenching of F_m, which is due, additionally, to electron acceptance and thus an increased rate of photochemistry. The effects of different concentrations (5–160 μM) of DCBQ on the Chl *a* fluorescence transient

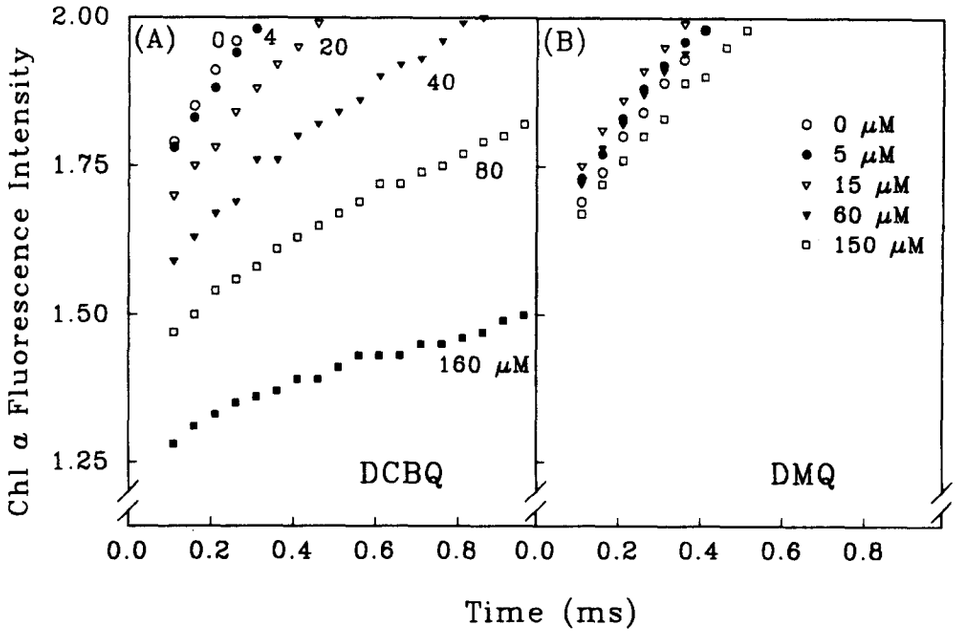

Fig. 2. Chl *a* fluorescence rise in the first 1 ms in the presence of different concentrations of DCBQ (A) and DMQ (B). Quinone concentrations are indicated in the figure. All the experimental conditions are the same as in Fig. 1 except that illumination was 300 W m^{-2} of 650 nm light.

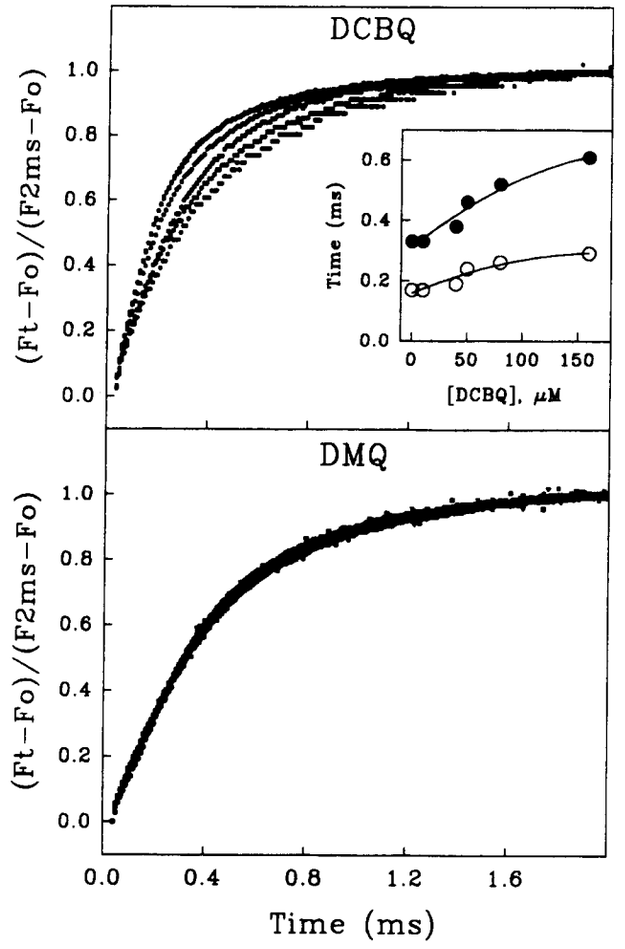

Fig. 3. Effects of DCBQ and DMQ on the variable Chl *a* fluorescence ($(F_t-F_o)/(F_{2\,ms}-F_o)$) induction curve in the first 2 ms (up to level J). Data were obtained from Fig. 1. Inset shows the one- half (open symbols) and three-quarters (filled symbols) rise times up to 2 ms in the presence of different DCBQ concentrations.

from spinach thylakoids excited with 600 W m^{-2} of 650 nm light are shown in Fig. 1 (top panel). For comparison, results obtained with DMQ are shown in the bottom panel of Fig. 1. In control samples, i.e. without exogenous quinones, the Chl *a* fluorescence curves (uppermost curves in each panel), plotted on a logarithmic time scale, start from the F_o level and increase to a maximum peak (P) with a clear step J (for complete and pronounced transients in algal cells and leaves, see Refs. [27] and [28]). In control thylakoid membranes, P levels off in about 300 ms at the intensity used. However, the major observations are: (1) a larger decrease in the variable (F_o to P) Chl *a* fluorescence by DCBQ than by DMQ and (2) a concentration-dependent decrease in F_o with DCBQ but not with DMQ. The decrease in F_o by DCBQ can be seen clearly even in the presence of DCMU (inset in Fig. 1, top panel). This decrease in F_o is most easily explained by quenching of antenna fluorescence, whereas that of F_m is additionally due to competition with electron acceptance.

Fig. 2 shows the concentration dependence of the effects of DMQ and DCBQ on the initial (up to 1 ms) Chl *a* fluorescence transient excited by 300 W m^{-2} of 650 nm light. DMQ at 5–60 μM does not decrease the F_o–J phase, but a small decrease in this phase is observed for 150 μM DMQ. In contrast, DCBQ in the 20–150 μM range lowers significantly not only the initial fluorescence rise F_o to J, but also the F_o fluorescence level. This clearly establishes the differential effects of DMQ and DCBQ on Chl *a* fluorescence transients, but it contradicts the conclusions of Cao and Govindjee [22]. However, it confirms the results of Lavergne and Leci [23]. On the other hand, the DMQ results confirm the observationss of both Cao and Govindjee [22] and Lavergne and Leci [23].

To visualize the effect of quinone strictly on the Chl *a* fluorescence induction kinetics up to the J level (the photochemical part; see Strasser et al. [27]), the data were plotted as $(F_t-F_o)/(F_{2\,ms}-F_o)$, where F_t is the fluorescence intensity at time *t*, up to 2 ms (Fig. 3). The results, in the doubly normalized plots, indicate that DCBQ slows down the rate of the Chl *a* fluorescence induction curve from F_o to level J (top panel), which is totally unaffected by DMQ (bottom panel).

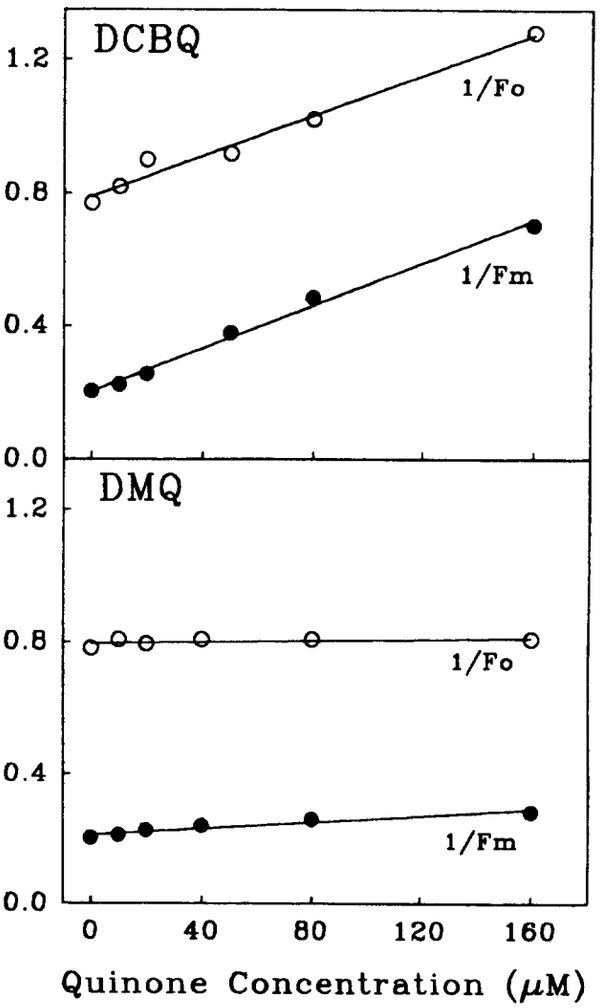

Fig. 4. Reciprocal of the initial (F_o) and maximum (F_m) fluorescence levels vs. the quinone concentration.

Table 1
Experimental values of the slopes obtained from the data in Fig. 4 in relative units (A). Calculated values of the Stern–Volmer constants K_D and F_o and F_m (B). Estimated concentrations of quencher needed ($1/K_D$) to decrease 50% of the fluorescence signals of F_o and F_m without quencher (C)

	Quencher	
	DCBQ	DMQ
(A)		
$\frac{1}{\Delta F_o \Delta[Q]} = \frac{^2k_q}{I_a k_F}$	3.03×10^{-3}	0.09×10^{-3}
$\frac{1}{\Delta F_m \Delta[Q]} = \frac{^2k_q}{I_a k_F}$	3.21×10^{-3}	0.47×10^{-3}
(B)		
$K_D(o) = \frac{^2k_q}{I_a k_F} F_o(Q=0)$	3.94×10^{-3} μM^{-1}	0.10×10^{-3} μM^{-1}
$K_D(m) = \frac{^2k_q}{I_a k_F} F_m(Q=0)$	15.72×10^{-3} μM^{-1}	2.36×10^{-3} μM^{-1}
(C)		
$\frac{1}{K_D(o)}$	254 μM	10000 μM
$\frac{1}{K_D(m)}$	64 μM	424 μM

The inset in Fig. 3 shows the one-half (open symbols) and three-quarters (filled symbols) rise times up to 2 ms in the presence of different concentrations of DCBQ. In the thylakoid membranes treated with 160 μM DCBQ, the three-quarters rise time is almost double that of the untreated thylakoids.

Stern–Volmer analyses [23,29–31] of the fluorescence data enable the quinone quenching constants to be determined (Fig. 4) for both the open (at F_o) and closed (at F_m) states of PSII RCs. The reciprocal values of the fluorescence signals in the presence of the quinones DCBQ and DMQ are shown in Fig. 4 as $1/F_o$ and $1/F_m$. In all situations, a straight line is found. This indicates that the data can be discussed in terms of the Stern–Volmer equation as follows [23,29–31]

$$\frac{F(Q=0) \text{ without quencher}}{F \text{ with quencher}} = 1 + \tau(Q=0)\times{}^2k_q\times[Q] \quad (1)$$

and

$$\tau(Q=0) = \frac{F(Q=0)}{I_a k_F} \quad (2)$$

where ^2k_q is the bimolecular (second-order) quenching constant (s^{-1} mol^{-1}), k_F is the first-order rate constant for fluorescence emission, I_a is the light absorption flux which excites the fluorescing pigments, [Q] is the concentration of the quencher and $\tau(Q=0)$ is the lifetime of the pigment fluorescing in the absence of the quencher. At F_o, all RCs are open and the lifetime is $\tau_o(Q=0) = F_o(Q=0)/I_a k_F$; at F_m, all RCs are closed and the lifetime is $\tau_m(Q=0) = F_m(Q=0)/I_a k_F$.

On the basis of Eqs. (1) and (2), the functions $1/F_o$ and $1/F_m$ vs. the quencher concentrations ([Q]) can be formulated as

$$\frac{1}{F_o} = \frac{1}{F_o(Q=0)} + \frac{^2k_q}{I_a k_F}[Q] \quad (3)$$

$$\frac{1}{F_m} = \frac{1}{F_m(Q=0)} + \frac{^2k_q}{I_a k_F}[Q] \quad (4)$$

Fig. 4 and Table 1 show that, in the presence of DCBQ, two parallel straight lines for $1/F_o$ and $1/F_m$ are obtained. This shows (according to Eqs. (3) and (4)) that DCBQ acts as a quencher for Chl *a* of the open and closed RCs with the same second-order quenching constant ^2k_q. In contrast, DMQ (Fig. 4) shows practically no quenching effect on the fluorescence of open RCs (F_o) and only a very small effect on closed RCs (F_m) at the concentrations used.

For comparison, the present data (Fig. 5(A)) and the data of Lavergne and Leci [23] (Fig. 5(B)) are plotted together. On the basis of the half rise time of Chl *a* fluorescence in the presence of DCMU, the light intensity used by Lavergne and Leci [23] was about 100 times lower than the light intensity

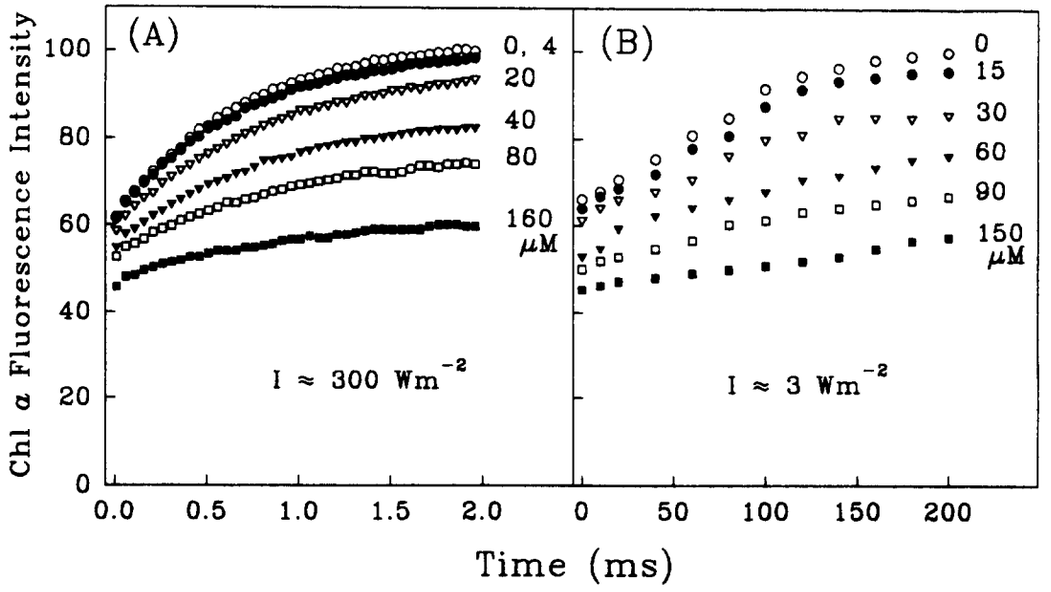

Fig. 5. (A) Effect of different concentrations of DCBQ on the Chl *a* fluorescence rise illuminated with 300 W m^{-2} light intensity. For comparison, a replot of the data of Lavergne and Leci [23] is shown (B). The abscissa for the present experiment with 300 W m^{-2} light intensity is expanded 100 times to adjust for the "intensity × time" equivalence of the photochemical reaction.

used in the results presented in Fig. 5(A). Considering the light dose (light intensity × time of the transient), the fluorescence rise times in Figs. 5(A) and 5(B) are very similar (note the different time axes in Figs. 5(A) and 5(B)). These data further verify that the quenching effect of DCBQ depends only on the quenching constant and the quinone concentration and is independent of the applied light intensity.

In order to explain the differences between the conclusions of this paper and those of Cao and Govindjee [22], we re-examined their crucial figures (Figs. 1(A) and 1(B)), and discovered that the replot of the 20 μM DMQ curve in Fig. 1(B) from Fig. 1(A) had an erroneous transposition of the F_o level. It should have been closer to the curve for the 2.5 μM DCBQ starting point than to the 20 μM DCBQ starting point. This unfortunate error seems to be the cause of the erroneous conclusion reached in that paper.

Table 2
Effect of different concentrations of DCBQ and DMQ on the rate of O_2 evolution by the thylakoid membranes. Thylakoids were suspended in the same buffer as described in Fig. 1, incubated for 5 min in the dark before the addition of quinones, and then exposed to 300 W m^{-2} of red (Schott RG 610) actinic light. Rate of O_2 evolution was measured up to 1 min after turning on the actinic light (± SD, $n = 3$)

[Quinones] (μM)	Rate of O_2 evolution (μmol O_2 (mg Chl)$^{-1}$ h^{-1})	
	DCBQ	DMQ
10	33.0 ± 2.6 [a]	13.2 ± 0.4
20	43.9 ± 3.5	34.9 ± 1.7
50	52.9 ± 3.5	41.5 ± 6.2
100	124.0 ± 17.6	63.2 ± 4.2
160	117.0 ± 7.6	65.0 ± 2.8
200	126.2 ± 14.6	76.1 ± 2.8

[a] Rate of O_2 evolution measured in the same experimental conditions, but in the presence of 2 mM ferricyanide, was 245 μmol O_2 (mg Chl)$^{-1}$ h^{-1}.

In order to confirm that, at the concentrations of quinones and light intensities used here, the expected and known effects on O_2 evolution were observed, we measured the Hill reaction rates with both DMQ and DCBQ using 300 W m^{-2} red light (see Table 2). Although both quinones acted as electron acceptors, DCBQ was more effective than DMQ, as expected (see, for example, Graan and Ort [15] and Nedbal et al. [21]). Furthermore, the rate of O_2 evolution measured in the presence of ferricyanide (2 mM), for the thylakoid used in the present work, was about 245 μmol O_2 (mg Chl)$^{-1}$ h^{-1}, showing that these thylakoids are comparable with those used by other investigators.

Our results show that DMQ suppresses the fluorescence rise from F_o to the P level less effectively than DCBQ. This is consistent with the higher rates of electron flow with DCBQ than with DMQ. This can be most easily explained by suggesting that DCBQ acts by relieving a blocking step involved in the re-oxidation of a fraction of the plastoquinol pool, as proposed by Lavergne and Leci [23]. An alternative explanation is that DCBQ prevents electron accumulation because it substitutes for PQ in the Q_B pocket, accepting electrons directly from Q_A^- [32], not only because it opens up the inactive PSII centers.

4. Concluding remarks

Although there is a clear differential sensitivity of DMQ and DCBQ on Chl *a* fluorescence transients and O_2 evolution, fluorescence data cannot be used to differentiate between the

so-called "active" and "inactive" (or slow) PSII centers, as was reported previously [22]. According to Lavergne and Leci [23], DCBQ is more effective than DMQ either because it re-oxidizes PQH_2 more effectively or replaces Q_B at the Q_B site. Another possibility is that the two quinones interact differently with the non-heme iron that lies between Q_A and Q_B [33]. Further experiments are needed to confirm whether or not DCBQ can accept electrons from inactive PSIIs and to understand the different mechanisms of interaction of DMQ and DCBQ with PSII antenna and RCs.

Acknowledgements

The authors thank Drs. Tasso Melis and Jerome Lavergne for their suggestions. This work was supported by the National Swiss Foundation grant 31-33678-92.

References

[1] O. Hansson and T. Wydrzynski, Current perceptions of photosystem II, *Photosynth. Res., 23* (1990) 131–162.

[2] Govindjee and M.R. Wasielewski, Photosystem II: from femtosecond to a millisecond, in W.R. Briggs (ed.), *Photosynthesis*, Alan R. Liss, New York, 1989, pp. 71–103.

[3] A.R. Crofts and C.A. Wraight, The electrochemical domain of photosynthesis, *Biochim. Biophys. Acta, 726* (1983) 149–185.

[4] B.A. Diner, V. Petrouleas and J. Wendoloski, The iron–quinone electron-acceptor complex of photosystem II, *Physiol. Plant., 81* (1991) 423–436.

[5] Govindjee, Photosystem II heterogeneity: the acceptor side, *Photosynth. Res., 25* (1990) 151–160.

[6] L. Nedbal and J. Whitmarsh, Photosystem 2 reaction centers inactive in plastoquinone pool reduction—a brief overview, *Photosynthetica, 27* (1992) 57–61.

[7] G. Papageorgiou, Chlorophyll fluorescence: an intrinsic probe of photosynthesis, in Govindjee (ed.), *Bioenergetics of Photosynthesis*, Academic Press, London, 1975, pp. 319–371.

[8] J. Lavorel and A.L. Etienne, In vivo chlorophyll fluorescence, *Topics in Photosynthesis, 2* (1977) 203–268.

[9] M.K. Joshi and P. Mohanty, Probing photosynthetic performance by chlorophyll *a* fluorescence: analysis and interpretation of fluorescence parameters, *J. Sci. Ind. Res., 54* (1995) 155–174.

[10] A. Melis and P.H. Homann, Kinetic analysis of the fluorescence induction in 3-(3,4-dichlorophenyl)-1,1-dimethylurea poisoned chloroplasts, *Photochem. Photobiol., 21* (1975) 431–437.

[11] A. Melis and P.H. Homann, Heterogeneity of the photochemical centers in system II of chloroplasts, *Photochem. Photobiol., 23* (1976) 343–350.

[12] J. Lavergne, Two types of primary acceptor in chloroplasts photosystem II. I. Different recombination properties, *Photobiochem. Photobiophys., 3* (1982) 257–271.

[13] J. Lavergne, Two types of primary acceptors in chloroplasts photosystem II. II. Reduction in two successive photoacts, *Photobiochem. Photobiophys., 3* (1982) 273–285.

[14] A. Melis, Functional properties of photosystem IIβ in spinach chloroplasts, *Biochim. Biophys. Acta, 808* (1985) 334–342.

[15] T. Graan and D.R. Ort, Detection of oxygen-evolving photosystem II centers inactive in plastoquinone reduction, *Biochim. Biophys. Acta, 852* (1986) 320–330.

[16] R.A. Chylla and J. Whitmarsh, Light saturation response of inactive photosystem II reaction centers in spinach, *Photosynth. Res., 25* (1990) 39–48.

[17] A. Melis, Dynamics of photosynthetic membrane composition and function, *Biochim. Biophys. Acta, 1058* (1991) 87–106.

[18] R.A. Chylla, G. Garab and J. Whitmarsh, Evidence for slow turnover in a fraction of photosystem II complexes in thylakoid membranes, *Biochim. Biophys. Acta, 894* (1987) 562–571.

[19] R.A. Chylla and J. Whitmarsh, Inactive photosystem II complexes in leaves. Turnover rate and quantitation, *Plant Physiol., 90* (1989) 765–772.

[20] J.E. Guenther, J.A. Nemson and A. Melis, Photosystem stoichiometry and chlorophyll antenna size in *Dunaliella salina* (green algae), *Biochim. Biophys. Acta, 934* (1988) 108–117.

[21] L. Nedbal, C. Gibas and J. Whitmarsh, Light saturation curves show competence of the water splitting complex in inactive photosystem II reaction centers, *Photosynth. Res., 30* (1991) 85–94.

[22] J. Cao and Govindjee, Chlorophyll *a* fluorescence transient as an indicator of active and inactive photosystem II in thylakoid membranes, *Biochim. Biophys. Acta, 1015* (1990) 180–188.

[23] J. Lavergne and E. Leci, Properties of inactive photosystem II centers, *Photosyn. Res., 35* (1993) 323–343.

[24] R.J. Porra, W.A. Thompson and P.E. Kriedemann, 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] J. Amesz and D.C. Fork, Quenching of chlorophyll fluorescence by quinones in algae and chloroplasts, *Biochim. Biophys Acta, 143* (1967) 97–107.

[26] M. Kitajima and W.L Butler, Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone, *Biochim. Biophys. Acta, 376* (1975) 105–115.

[27] R.J. Strasser, A. Srivastava and Govindjee, Polyphasic chlorophyll *a* fluorescence transient in plants and cyanobacteria, *Photochem. Photobiol., 61* (1995) 32–42.

[28] A. Srivastava, R.J. Strasser and Govindjee, Polyphasic rise of chlorophyll *a* fluorescence in herbicide-resistant D1 mutants of *Chlamydomonas reinhardtii*, *Photosynth. Res., 43* (1995) 131–141.

[29] S.S. Lehrer, Solute perturbation of protein fluorescence. Quenching of the tryptophyl fluorescence of model compounds and of lysozyme by iodine ion, *Biochemistry 10* (1971) 3254–3263.

[30] J.R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Plenum, New York, 1984, Chapter 9.

[31] K.K. Karukstis, R.M. Moision, S.K. Johansen, K.E. Birkeland and S.M. Cohen, Alternative measure of photosystem II electron transfer inhibition in anthroquinone-treated chloroplasts, *Photochem. Photobiol., 55* (1992) 125–132.

[32] J. Lavergne, Improved UV–visible spectra of the S-transitions in the photosynthetic oxygen-evolving system, *Biochim. Biophys. Acta, 1060* (1991) 175–188.

[33] H. Koike, Y. Kashino and K. Satoh, Interaction of halogenated benzoquinones with the non-heme ions (Q400) in photosystem II, *Z. Naturforsch., Teil C, 48* (1993) 168–173.