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Biochimica et Biophysica Acta 1604 (2003) 115–123
BIOCHIMICA ET BIOPHYSICA ACTA
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Interaction of exogenous quinones with membranes of higher plant chloroplasts: modulation of quinone capacities as photochemical and non-photochemical quenchers of energy in Photosystem II during light–dark transitions

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Received 31 December 2002; received in revised form 26 March 2003; accepted 28 March 2003

Abstract

Light modulation of the ability of three artificial quinones, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB), 2,6-dichloro-*p*-benzoquinone (DCBQ), and tetramethyl-*p*-benzoquinone (duroquinone), to quench chlorophyll (Chl) fluorescence photochemically or non-photochemically was studied to simulate the functions of endogenous plastoquinones during the thermal phase of fast Chl fluorescence induction kinetics. DBMIB was found to suppress by severalfold the basal level of Chl fluorescence (F_o) and to markedly retard the light-induced rise of variable fluorescence (F_v). After irradiation with actinic light, Chl fluorescence rapidly dropped down to the level corresponding to F_o level in untreated thylakoids and then slowly declined to the initial level. DBMIB was found to be an efficient photochemical quencher of energy in Photosystem II (PSII) in the dark, but not after prolonged irradiation. Those events were owing to DBMIB reduction under light and its oxidation in the dark. At high concentrations, DCBQ exhibited quenching behaviours similar to those of DBMIB. In contrast, duroquinone demonstrated the ability to quench F_v at low concentration, while F_o was declined only at high concentrations of this artificial quinone. Unlike for DBMIB and DCBQ, quenched F_o level was attained rapidly after actinic light had been turned off in the presence of high duroquinone concentrations. That finding evidenced that the capacity of duroquinone to non-photochemically quench excitation energy in PSII was maintained during irradiation, which is likely owing to the rapid electron transfer from duroquinol to Photosystem I (PSI). It was suggested that DBMIB and DCBQ at high concentration, on the one hand, and duroquinone, on the other hand, mimic the properties of plastoquinones as photochemical and non-photochemical quenchers of energy in PSII under different conditions. The first model corresponds to the conditions under which the plastoquinone pool can be largely reduced (weak electron release from PSII to PSI compared to PSII-driven electron flow from water under strong light and weak PSI photochemical capacity because of inactive electron transport on its reducing side), while the second one mimics the behaviour of the plastoquinone pool when it cannot be filled up with electrons (weak or moderate light and high photochemical competence of PSI).

Keywords: Chlorophyll fluorescence quenching; Quinone; Photosystem II; Thylakoid

1. Introduction

Quinones are ubiquitous to photosynthetic membranes. In thylakoids of higher plants, plastoquinones operate as key mobile electron carriers functionally connecting Photosystem II (PSII) and Photosystem I (PSI) [1]. Their number exceeds by severalfold that of PSII reaction centers thus forming a specific pool. On the reducing side of PSII, electrons are transferred from a bound plastoquinone molecule, Q_A, to a two-electron plastoquinone acceptor, known

Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCBQ, 2,6-dichloro-*p*-benzoquinone; duroquinone, tetramethyl-*p*-benzoquinone; Chl, chlorophyll; F_o, basal level of chlorophyll fluorescence; F_m, maximum level of chlorophyll fluorescence; F_v, variable fluorescence; K_{SV}, Stern–Volmer quenching constant; PSII and PSI, Photosystem II and Photosystem I; Q_A and Q_B, primary and secondary quinone acceptors of Photosystem II

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doi:10.1016/S0005-2728(03)00042-2

as Q_B. Double reduction releases Q_B from its binding site on the polypeptide D1 of PSII reaction center. Diffusion and binding to that site of a new oxidized molecule from the plastoquinone pool maintain the photochemical competence of PSII.

A dual function is ascribed to plastoquinones as they act as both photochemical and non-photochemical quenchers of energy in PSII. Indeed, in addition to their involvement into photosynthetic electron transport, plastoquinones contribute to non-photochemical control of excitation energy dynamics in light-harvesting antenna. Oxidized plastoquinones were found to reduce the chlorophyll (Chl) fluorescence yield in vivo by 5–20% [2–5]. Reduction of the plastoquinone pool removes that quenching. This was proposed to occur during the thermal phase of Chl fluorescence rise observed under strong light [6–8]. A widely spread approach used to examine the functions of the plastoquinone pool is the introduction of artificial substituted quinones into the thylakoid membrane. Exogenously added quinones are able to compete with plastoquinone for electrons thus inhibiting electron transfer from PSII to PSI [9–11]. In this respect, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) [12,13], 2,6-dichloro-*p*-benzoquinone (DCBQ) [14–17], and tetramethyl-*p*-benzoquinone (duroquinone) are the most efficient inhibitors of cytochrome b_6/f reduction by plastoquinol. The mode of action of artificial quinones on electron transport activity in PSII is thought to be the displacement of plastoquinone from the Q_B binding site [17,18]. As the concentration of exogenous quinones in the thylakoid membrane can exceed to a large extent that of endogenous plastoquinones, they often accelerate Q_A^- oxidation with a corresponding decrease in variable Chl fluorescence (F_v) [14,16].

Artificial quinones are efficient quenchers of Chl fluorescence in organic solutions [19]. Several authors have shown that such quinones can also quench excited singlet states of antenna Chl molecules in higher plants and green bacteria thus decreasing basal Chl fluorescence level (F_o) [18,20–24]. The degree of Chl fluorescence quenching is highly variable and depends on quinone structure [23]. Thus, similarly to plastoquinone-dependent Chl fluorescence quenching, the quenching by artificial quinones is a consequence of both non-photochemical mechanism due to direct interaction between the added quinones and the excited antenna Chls, and photochemical events owing to competition between artificial and natural quinones for electrons injected by PSII.

As artificial quinones are PSII electron acceptors, they can be partially (or even completely) reduced under illumination with concomitant loss of their ability to act as both non-photochemical and photochemical quenchers. Thus, the behaviour of artificial quinones during dark–light–dark transitions is expected to mimic that of endogenous plastoquinones, which can be reversibly reduced under light [25,26]. The above hypothesis was tested in this work. We examined three quinones that are frequently used in the studies of photosynthetic electron transport, DBMIB, DCBQ, and duroquinone. They have different redox properties. Unlike DBMIB and DCBQ, which only act as PSII electron acceptors, duroquinone is also able to effectively shuttle electrons from PSII to PSI being reduced by PSII and oxidized by the cytochrome b_6/f complex. DBMIB and DCBQ demonstrated the expected response, reversibly losing both non-photochemical and photochemical quenching capacities under light with further restoration of these properties in the dark. In contrast, duroquinone exhibited a different mode of action explained by the high efficiency of duroquinol to donate reducing equivalents to PSI thus preventing the exhaustion of the pool of oxidized artificial quinone under light. A clear separation between photochemical and non-photochemical quenching properties of the added quinones was demonstrated under dynamic conditions of electron transport.

2. Materials and methods

Thylakoids were isolated from freshly harvested leaves of pea (*Pisum sativum* L.) grown in a climatic chamber under light provided by a xenon lamp at a fluence rate of 200 μmol quanta m^{-2} s^{-1}. Plants were cultivated in soil. Cooled leaves were ground in a medium containing 100 mM sorbitol, 20 mM Hepes–NaOH, 10 mM NaCl, and 2 mM $MgCl_2$. The suspension was filtered through four layers of nylon cloth and centrifuged at $150 \times g$ for 5 min at 4 °C. The supernatant was further centrifuged at $350 \times g$ for 10 min at 4 °C. The pellet was resuspended in a medium containing 20 mM Hepes–NaOH, 10 mM NaCl, and 2 mM $MgCl_2$.

Chl was assayed according to Porra et al. [27]. The concentration of Chl during the measurements was 50 μg ml^{-1}.

Stock solutions of the quinones were prepared in distilled water (DBMIB) or in ethanol (duroquinone and DCBQ). Quinone-enriched thylakoids were prepared by adding given volumes of quinone stock and buffer solutions. Before the measurements, thylakoids were incubated with quinones for 2 min in the dark.

Chl fluorescence was measured with a PAM Chl fluorometer (Walz, Effeltrich, Germany) in glass cuvettes with an optical pass of 1.065 mm. The signals were recorded with a computer using the PAM Data Acquisition System PDA-100. The sampling rates were 1, 3, or 10 ms/point. White actinic light was obtained from a Fiber-Lite light source (Microview, Thornhill, Canada) and controlled by an electronic shutter.

3. Results

Fig. 1 shows the original traces of Chl fluorescence transients induced by white actinic light in isolated thyla-

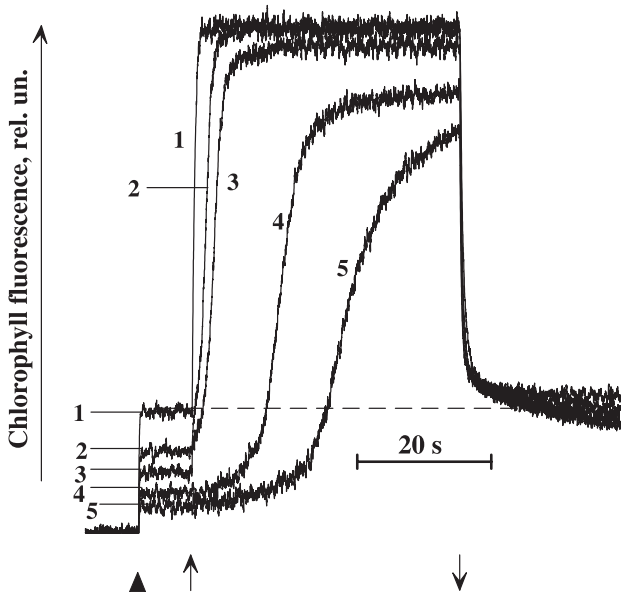

Fig. 1. Original traces of Chl fluorescence transients induced by irradiation of a suspension of isolated thylakoids with white light of 175 W m^{-2} in the absence of additives (1) or in the presence of 30 (2), 45 (3), 80 (4), or 115 (5) μM DBMIB. Here, and in other figures, upward triangles indicate the onset of weak excitation light; upward and downward arrows indicate actinic light on and off, respectively. Sampling rate was 3 ms/point.

koid membranes supplied with various concentrations of DBMIB. Chl fluorescence during dark–light–dark transitions was influenced by DBMIB in several characteristic ways. The first clear effect was a progressive quenching of F_o as DBMIB concentration increased. This is in agreement with data from numerous reports cited above. Fig. 2 shows that the extent of F_o quenching increased sharply with DBMIB concentration. This concentration dependence was fitted according to the Stern–Volmer equation, which describes the static quenching of Chl fluorescence by exogenous quinones [28]:

$$F_o/F'_o = 1 + K_{SV}[Q],$$

where F_o and F'_o are the observed F_o levels in the absence and presence of added quinone, respectively; K_{SV} is the Stern–Volmer quenching constant; and [Q] is the concentration of added quinone. A linear Stern–Volmer plot of F_o/F'_o vs. [Q], as exhibited by DBMIB in Fig. 2, constitutes an indication of uniform Chl fluorophores with equal accessibility to the quencher. The obtained value for K_{SV} was 4.13×10^4 M^{-1}.

A second action of DBMIB was a marked attenuation of the light-induced rise of variable Chl fluorescence to the maximum level (F_m), and a transformation of its kinetics from apparently monotonous to sigmoidal (Fig. 1). It should be noted that with greater time resolution, a sigmoidal trace is also obtained for untreated pea thylakoids [29]. Also, the F_m level was significantly reduced. In control samples, the half-rise time of the fluorescence induction curve was 265 ms. The above effects are characterized by an increasing area above the kinetic curve. A nonmonotonous relationship was found between this area and DBMIB concentration (Fig. 2).

In DBMIB-treated samples, after actinic light had been turned off, Chl fluorescence relaxed to a higher level compared to F_o level observed with DBMIB before irradiation (Fig. 1). This level of Chl fluorescence rapidly attained after cessation of actinic light irradiation did not depend on the concentration of DBMIB and was similar to F_o level in untreated thylakoids. However, in contrast with control samples, this initial rapid phase of Chl fluorescence relaxation in DBMIB-treated thylakoids was followed by a much slower decline (Fig. 1). The latter lasted for several minutes until the F_o level observed before irradiation by actinic light was finally attained thus demonstrating full reversibility of the light-induced changes (result not shown).

Fig. 3 exhibits the transformations in the kinetics of F_v dark relaxation depending on the irradiation period in DBMIB-treated thylakoids. F_v decayed rapidly if actinic light exposure was short (Fig. 3A). Only a fast exponentially relaxing component with half-time of 18–19 ms was found in the kinetics of F_v dark decay measured after such relatively short irradiation (3 s) of isolated thylakoids in the presence of DBMIB (Fig. 3B). That component is due to fast Q_A^- reoxidation. However, complex kinetics of F_v relaxation consisting of slow (51% of total F_v, half-time of 340 ms), middle (33%, 58 ms), and fast (17%, 14 ms) exponentially decaying components were found if actinic light was turned off after Chl fluorescence had attained its maximum level (7 s) (Fig. 3C). The appearance of middle and slow components indicated that Q_A^- reoxidation was restricted [30]. The contribution from middle and slow components into the kinetics of F_v relaxation increased with the duration of actinic light.

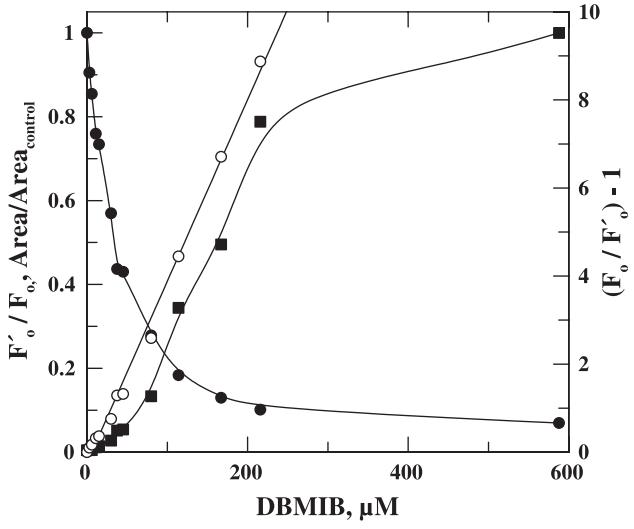

Fig. 2. Influence of DBMIB concentration on the quenching of F_o presented in usual (dark circles) or Stern–Volmer (light circles) plots and on the area above the curve of light-induced F_v rise (squares).

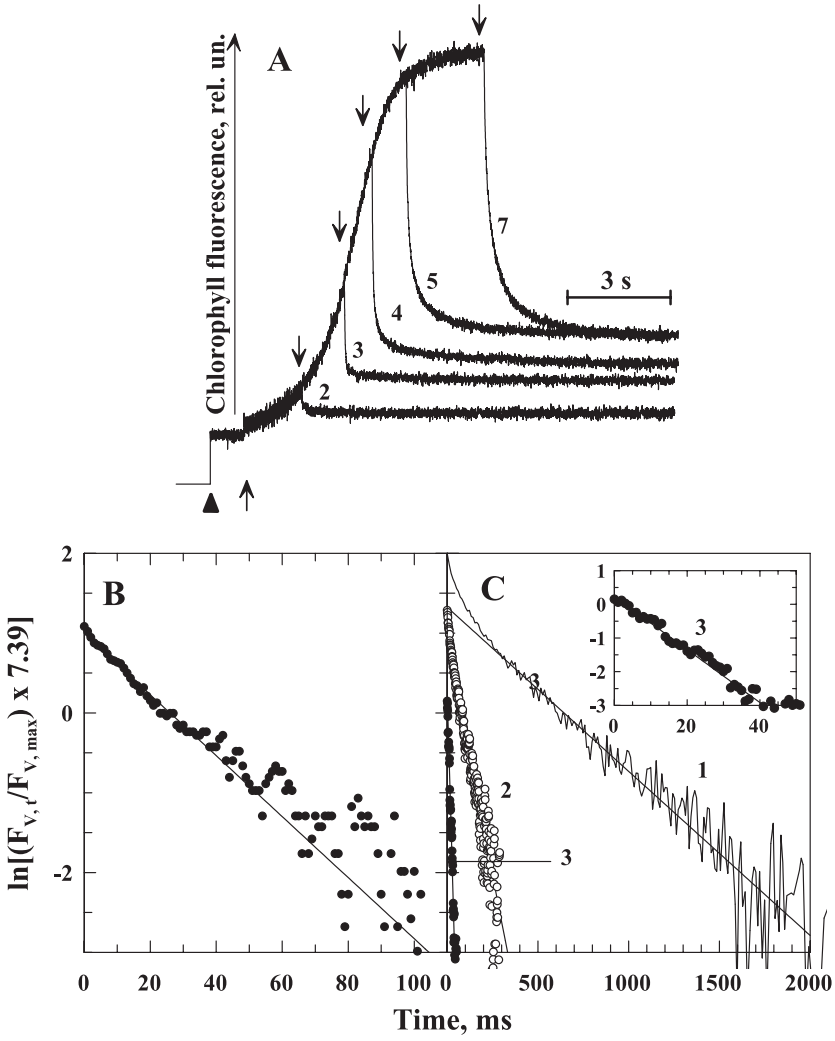

Fig. 3. (A) Kinetics of F_v relaxation in the dark after various periods of irradiation (in seconds, as indicated by numbers adjacent to traces) by white light of 175 W m^{-2} in the presence of 50 μM DBMIB. (B) Semilogarithmic plot of F_v dark decay observed as in A after a 3-s irradiation and (C) after a 7-s irradiation. In C, trace 1 represents the original kinetics trace including all three components and traces 2 and 3 are the deconvoluted middle and fast kinetic components, respectively; inset: the fast component of trace 3 with extended time scale. Sampling rate was 1 ms/point. Each measurement was done with a new sample.

The sigmoidal kinetics of light-induced F_v rise observed in DBMIB-treated thylakoids slowly reverted in the dark to the monotonous kinetics observed in untreated thylakoids (Fig. 4). We quantified the time course of the latter process using as parameter the area above the kinetic curve of F_v rise as depicted in Fig. 4 (inset). Such areas were measured during the initial irradiation and also during a following irradiation given after various periods of dark incubation between the two light exposures. Then, areas obtained for the second irradiations were normalized to those found for the first irradiations. The semilogarithmic plot of that ratio vs. time indicates that the restoration of the area above the kinetic curve of light-induced F_v rise proceeded according to a first-order reaction with a half-time of about 210 s (Fig. 4).

The most plausible explanation of the DBMIB-induced attenuation of F_v rise is that the artificial quinone molecules acted as electron acceptor for PSII thus retarding Q_A reduction. The reduced form of DBMIB being an inefficient fluorescence quencher, the ability of this quinone to quench Chl fluorescence declined with illumination, which finally allowed the emission of variable fluorescence after a prolonged period of illumination. The restriction of PSII photochemical capacity by some other treatments or the presence of electron acceptors other than quinones should prevent DBMIB reduction. Indeed, the ability of actinic light to induce F_v in DBMIB-treated thylakoids was found to be partially or even completely lost in the presence of redox agents or inhibitors of photosynthetic electron transport (Fig. 5). Addition of 17 μM TMPD, an acceptor of electrons from PSII, on the background of DBMIB significantly retarded the light-induced increase of variable Chl fluorescence and somewhat decreased F_m level (Fig. 5, traces 1 and 2). Those effects were more pronounced at higher (83 μM) TMPD concentration (Fig. 5, trace 3). Importantly, 83 μM

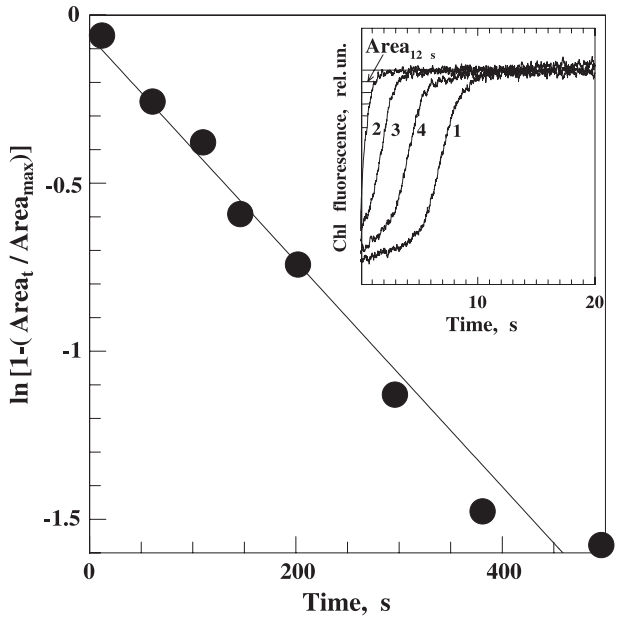

Fig. 4. Semilogarithmic plot of the time course of dark restoration of the area over the curve of F_v rise measured as indicated in inset. Inset: The kinetics of F_v rise during the first irradiation of dark-adapted thylakoids (1) or during the second irradiations starting 12 s (2), 60 s (3), or 200 s (4) after the first light exposure. Sampling rate was 10 ms/point. Each measurement was done with a new sample. DBMIB concentration was 50 μM. For other details, see Fig. 3.

TMPD given alone decreased F_m level by less than 10% and did not initiate changes in the kinetics of light-induced F_v rise (data not shown). CCCP, a well-known ADRY-agent, a substance which inactivates the higher "S" states of the water oxidation complex [31], as well as ferricyanide, completely abolished the appearance of light-induced F_v at high concentrations when added together with DBMIB (Fig. 5, traces 4–6). Similarly to TMPD, those substances added alone did not suppress F_v despite a small decrease in F_m. Addition of diuron, which blocks electron transfer from primary to secondary endogenous acceptors of PSII, completely suppressed light-induced F_v in the presence of DBMIB (Fig. 5, traces 7 and 8). Thus, the above data clearly revealed that both photochemical and non-photochemical DBMIB-dependent quenching of Chl fluorescence are reversibly lost during irradiation of isolated thylakoids. This phenomenon can proceed only under conditions of intense electron flow from PSII to the quinone.

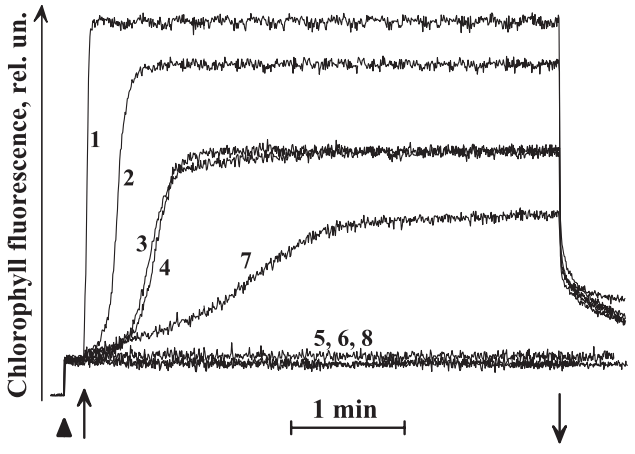

Fig. 5. Original traces of Chl fluorescence transients induced by white light of 175 W m^{-2} in the presence of 50 μM DBMIB (trace 1) or various chemicals on the background of 50 μM DBMIB: 17 (trace 2) or 83 (trace 3) μM TMPD; 60 (trace 4) or 600 (trace 5) μM CCCP; 0.6 mM ferricyanide (trace 6); 3 (trace 7) or 15 (trace 8) μM diuron. Sampling rate was 10 ms/point.

Fig. 6 shows the action of two other quinones, duroquinone and DCBQ, on F_o and light-induced F_v. These quinones altered the fluorescence properties of PSII differently compared to the action of DBMIB. Increasing concentrations of both duroquinone and DCBQ resulted in a progressive decrease in F_m with no initial effect on F_o. A quenching of F_o level was observed only at high concentrations of this quinone. A quantitative examination of the effect of various concentrations of duroquinone and DCBQ on F_o is presented in Fig. 7 using both usual and Stern–Volmer plots. A close similarity was found between the action of duroquinone and DCBQ on F_o level: significant quenching was observed only

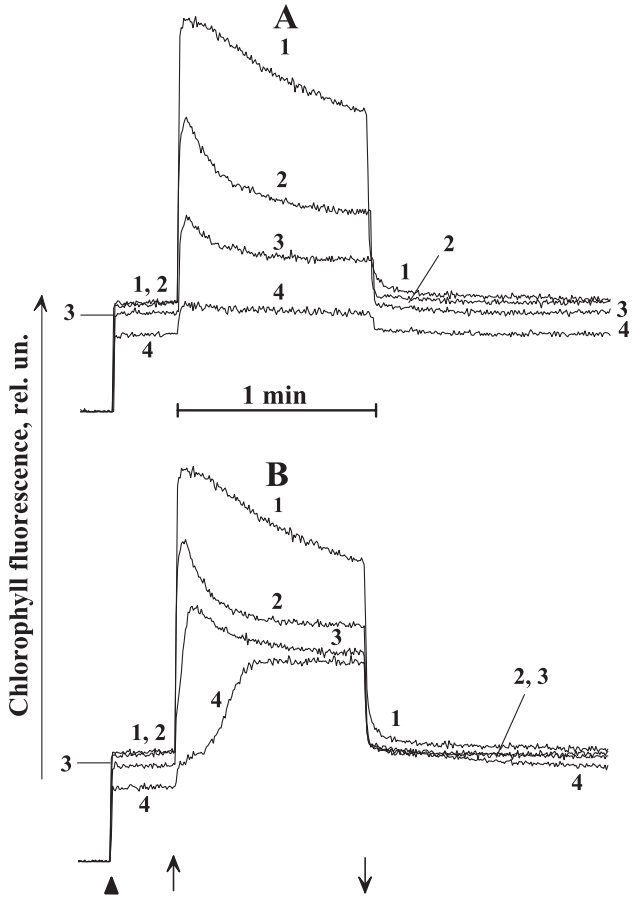

Fig. 6. Original traces of Chl fluorescence transients induced by white light of 175 W m^{-2} in the absence of additives (traces 1), or (A) in the presence of either 1.5 μM (trace 2), 50 μM (trace 3), or 300 μM (trace 4) duroquinone or (B) 58 μM (trace 2), 150 μM (trace 3), or 300 μM (trace 4) DCBQ. Sampling rate was 10 ms/point.

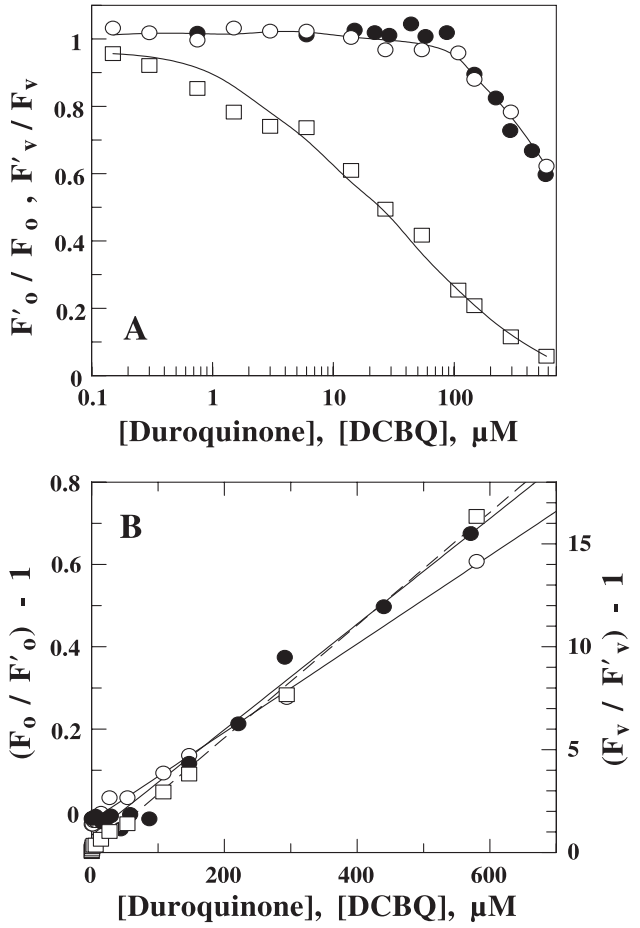

Fig. 7. Dependencies of F_o level (circles) or F_v extent (squares) on duroquinone (open symbols) or DCBQ (closed symbols) concentration presented as usual (A) or Stern–Volmer (B) plots. Both F_o and F_v were normalized to the values in untreated thylakoids.

at concentrations above 100 μM. Stern–Volmer quenching constants for F_o level were 1.05×10^3 M^{-1} and 1.67×10^3 M^{-1} for duroquinone and DCBQ, respectively.

Duroquinone and DCBQ significantly differed in their action on F_v. Addition of duroquinone at any concentration studied did not alter the kinetics of light-induced changes of variable fluorescence, which rapidly rose to a maximum after actinic light had been turned on (Fig. 6A). However, F_m decreased with increasing concentration of duroquinone (Fig. 6B), and, consequently, F_v also declined (Fig. 7A). Clearly, the action of duroquinone on the variable part of Chl fluorescence was more severe compared to the action on F_o; the Stern–Volmer quenching constant for F_v was 2.75×10^4 M^{-1} (Fig. 7B). In marked contrast to duroquinone, additions of DCBQ at high concentration initiated sigmoidal-type kinetics of light-induced F_v rise (Fig. 6B). In this respect, the action of DCBQ on variable Chl fluorescence was similar to that of DBMIB (see Fig. 1).

Another important difference between the changes initiated by duroquinone and DCBQ was the pattern of Chl fluorescence relaxation after actinic light was turned off. In duroquinone-treated thylakoids, fluorescence rapidly dropped down in the dark exactly to the initial F_o value (Fig. 6A). That was found irrespective of whether F_o was quenched by the addition of duroquinone or not. In DCBQ-treated thylakoids, rapid relaxation of Chl fluorescence to the initial F_o value was found only at the low concentrations of quinone that were insufficient to quench F_o (Fig. 6B). At high concentration of DCBQ, Chl fluorescence rapidly dropped down after prolonged irradiation to the level corresponding to F_o in untreated thylakoids. Further dark incubation of DCBQ-treated thylakoids was required to observe a slow decline in Chl fluorescence (Fig. 6B) similarly to that obtained for DBMIB (see Fig. 1).

Figs. 8 and 9, which present the semilogarithmic plots of F_v dark decay kinetics measured in duroquinone- and DCBQ-treated thylakoids, illustrate the capacities of those quinones as photochemical quenchers of energy in PSII.

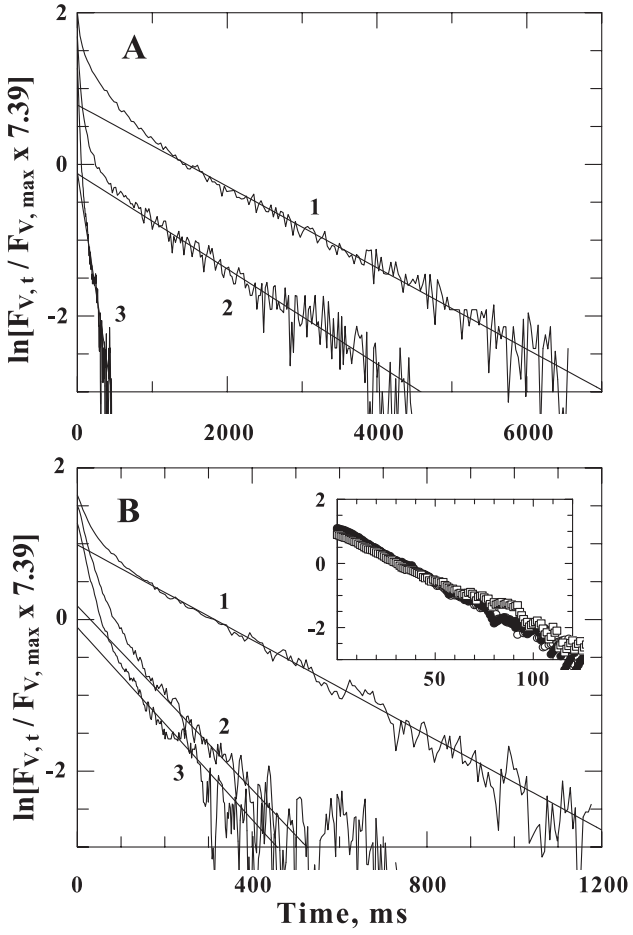

Fig. 8. Semilogarithmic plots of dark relaxation of F_v after 1-s irradiation by white actinic light of 175 W m^{-2} in isolated thylakoids without additive (trace 1 or open squares in inset) or in the presence of 0.6 (trace 2 or closed circles in inset) or 3 (trace 3 or open circles in inset) μM duroquinone. (A) Semilogarithmic plots of the time courses of original curves of F_v decay. (B and inset to panel B) Deconvoluted middle and fast components of F_v dark decay, respectively. Here, and on Fig. 9, F_v values were normalized to F_v in untreated thylakoids; lines represent linear fits for the corresponding kinetic components, and sampling rate was 1 ms/point.

Addition of duroquinone at concentrations as low as 0.6 μM was already sufficient to significantly decrease the slow and the middle components of F_v relaxation, whereas F_m was barely affected (Fig. 8). At higher concentration of duroquinone (3 μM), the slow component completely disappeared (Fig. 8A), and the magnitude of the middle one was declined by severalfold when compared to untreated thylakoids (Fig. 8B). The above findings indicate that Q_A^- reoxidation was greatly accelerated by the addition of this quinone to isolated thylakoids. Importantly, kinetic curves of F_v dark decay were similar in duroquinone-treated thylakoids after 1- and 60-s irradiations with actinic light (data not shown). Those data support the high capacity of duroquinone as photochemical quencher of PSII.

Similarly to duroquinone, DCBQ was found to accept electrons from PSII efficiently, which is obvious from the reduced magnitudes of the slow and middle components, respectively, of F_v dark decay in the presence of 58 μM DCBQ (Fig. 9A and B, trace 2). At higher concentration of this quinone, these kinetic components completely disappeared if F_v relaxation was measured after short actinic light irradiation. In that case, F_v decay was fitted solely by the fast component (inset to Fig. 9B, trace 3). Importantly, both middle and slow components appeared again in the kinetics of F_v relaxation if it was measured after a 60-s irradiation with actinic light, which caused an increase in F_m (see Fig. 9).

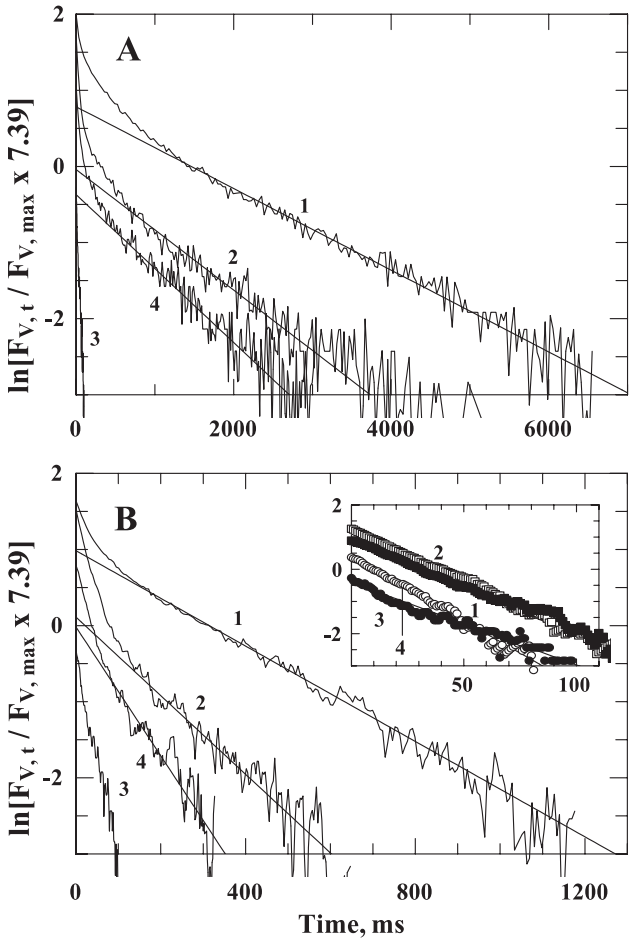

Fig. 9. Semilogarithmic plots of dark relaxation of F_v after irradiation by white actinic light of 175 W m^{-2} without additive (trace 1) or in the presence of 58 (trace 2) or 150 (traces 3 and 4) μM DCBQ. Periods of irradiation were 1 s (traces 1, 2 and 3) or 60 s (traces 4). (A) Semilogarithmic plots of the original time courses of F_v; (B) and inset to panel B) Deconvoluted middle and the fast components of F_v, respectively.

4. Discussion

Each of the three exogenous quinones used here altered the photochemical activity of PSII in a rather specific manner affecting strongly either the efficiency of excitation energy migration or electron transport. However, the observed quenching of F_o level of Chl fluorescence is well described by the Stern–Volmer equation for all artificial quinones examined in this work, which is consistent with previously reported data concerning various substituted artificial quinones [23]. A linear Stern–Volmer plot of F_o/F'_o vs. [Q], as exhibited by DBMIB (Fig. 2A), duroquinone, and DCBQ (Fig. 7), indicates uniform quenching of Chl fluorescence. DBMIB was shown to be an especially effective quencher of excitation energy absorbed by the light-harvesting antenna as revealed by dramatic quenching of F_o level of Chl fluorescence (see Fig. 1). Vasil'ev et al. [24] have shown that the decrease in F_o observed in the presence of 5-hydroxy-1,4-naphthoquinone was due to non-photochemical quenching of excitons in PSII antenna. Obviously, the same is true for the artificial quinones studied here.

Duroquinone exhibited high capacity as PSII electron acceptor, decreasing the extent of F_v even at low concentration, where it was not accompanied by non-photochemical antenna quenching (Fig. 6A). In addition, a significant difference found between the action of DBMIB and high concentrations of duroquinone on PSII photochemistry is that in the presence of duroquinone, Chl fluorescence rapidly relaxed to the initial quenched level of F_o when actinic light was turned off, while it declined to a level similar to F_o in untreated sample in DBMIB-treated thylakoids (compare Figs. 1 and 6). In other words, non-photochemical quenching reversed very slowly in the dark in the presence of DBMIB and very quickly (if it was changed at all by light) in duroquinone-treated thylakoids. Understanding the origin of those differences seems to be important to evaluate which quinone simulates better the dual functions of plastoquinones in thylakoid membranes.

The data presented here indicate that irradiation of DBMIB-treated thylakoids strongly suppressed the ability of this quinone to act as non-photochemical and photochemical quencher, which is determined by DBMIB photoreduction. The latter is obvious from the finding that PSII acceptors (other than quinones) that compete with DBMIB for electrons, and diuron, are able to prevent F_v quenching and the light-induced reversion of F_o in the presence of DBMIB (see Fig. 5). An important result is that light-

induced reversion of F_o quenching in the presence of DBMIB was, in turn, slowly abolished in the dark. After a rapid relaxation of F_v owing to Q_A^- reoxidation, Chl fluorescence slowly decreased to the previously observed quenched F_o. Both reoxidation of photoreduced DBMIB molecules within the thylakoid membrane or their exchange with oxidized quinones from outer medium could, in principle, account for the slow F_o decrease after the actinic light was turned off. However, the same changes were found during dark–light–dark transitions if thylakoids treated with DBMIB were washed twice from external quinones before measurement (data not shown). Thus, we can conclude that the slow decline of F_o was due to reoxidation of photoreduced DBMIB.

Importantly, the primary steps of the photo-induced restoration of F_o to a level similar to that in untreated thylakoids did not coincide with the loss of DBMIB ability to act as a photochemical quencher in PSII. This conclusion is supported by the finding that only small relaxation of Chl fluorescence quenching, that is, F_v appearance, was observed after short exposure of DBMIB-treated thylakoids to actinic light, which was already sufficient to remove more than 20% of F_o quenching (Fig. 3). Reversion of quenched F_o by one half corresponded to the development of only 25% of maximum F_v (Fig. 3). In the latter case, the kinetics of F_v dark relaxation was represented solely by the fast component, which indicates a highly oxidized plastoquinone pool [30]. This confirms the high efficiency of DBMIB as a photochemical quencher at the initial steps of the time course of the light-induced reversion of F_o quenching despite the partial photoreduction of the added quinone.

Thus, higher concentrations of oxidized DBMIB are required to efficiently quench energy in the antenna compared to that required to effectively accept electrons from PSII and keep Q_A in oxidized state under light. The same conclusion is even more obvious for duroquinone and DCBQ, which are much less efficient quenchers of excitons in PSII (see Fig. 7). Duroquinone and DCBQ are able to reduce F_v at low concentration owing to an effective release of electrons from Q_A^-, while they do not promote F_o quenching.

The difference between the behaviour of quenched F_o level during dark–light–dark transitions in the presence of either DBMIB or high concentrations of DCBQ, on one hand, and duroquinone, on the other hand, are likely related to the redox properties of reduced duroquinone. Duroquinol is known to be as an efficient electron donor to the cytochrome b_6/f complex [12]. Thus, under illumination that simultaneously excites both PSII and PSI, the rapid turnover of oxidized and reduced form of that quinone can prevent the exhaustion of the pool of duroquinone in the thylakoid membrane where oxygen acts as terminal electron acceptor.

As mentioned above, the photochemical quenching of energy in PSII promoted by artificial quinones is usually ascribed to the occupation of the Q_B binding site by the added quinone instead of endogenous plastoquinones [23]. In this respect, the finding that both duroquinone and DCBQ did not accelerate the fast component of F_v dark relaxation (see insets to Figs. 8 and 9) is of significant interest. This kinetic component was assumed to reflect the averaged rate of diffusion of an oxidized plastoquinone molecule to Q_B binding site [30,32]. As the concentration of added quinones is expected to be much larger than endogenous plastoquinones, the rate of the fast component of Q_A^- reoxidation was expected to be accelerated in the presence of DBCQ or, particularly, duroquinone. This was not observed in our experiments. An alternative explanation for the unchanged rate of the fast component is that substituted quinones do not interact directly with Q_A^-. Instead, they may oxidize plastohydroquinol molecules, as already known for various pairs of quinones [33], thus permanently keeping the pool of plastoquinones in oxidized state.

In summary, the data presented above clearly demonstrate the potential ability of exogenous quinones introduced into the thylakoid membrane to exhibit reversible changes of their capacities to act as photochemical and non-photochemical quenchers during dark–light–dark transitions. Those changes are, in principle, similar to those ascribed to endogenous plastoquinones. For either artificially introduced quinones or endogenous plastoquinones, those changes are determined by the turnover of the reduced and oxidized forms. The ability of mobile non-bound plastoquinone to act as photochemical quenchers of energy in PSII demonstrating reversible cycles of light-induced reduction and dark oxidation is a common knowledge [34]. Static non-photochemical quenching of Chl fluorescence was removed during I_2–P phase of F_v rise under strong light because of plastoquinone reduction [7,8,35]. The present study revealed that the behaviour of artificial plastoquinones during dark–light–dark transitions can be illustrated by two different models. One is represented by DBMIB or high concentration of DCBQ and the second by duroquinone. In a forthcoming paper, we will report that the behaviour of the plastoquinone pool during dark–light–dark transitions follows either the first or second model depending on conditions. Under condition, that favour plastoquinone pool in reduced state (strong light and relatively weak photochemical capacity of PSI owing to restricted electron flow on its reducing side), DBMIB and high concentrations of DCBQ well simulate the light-induced changes in the plastoquinone pool redox state. Oppositely, duroquinone represents an adequate model of the plastoquinone pool for the conditions under which the plastoquinone pool cannot be significantly reduced (weak or moderate light and high photochemical competence of PSI).

Acknowledgements

The authors acknowledge the financial support by Natural Sciences and Engineering Research Council of Canada.

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