Quenching of excited states of chlorophyll molecules in submembrane fractions of Photosystem I by exogenous quinones

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Abstract

The ability of three substituted quinones, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB), 2,6-dichloro-p-benzoquinone (DCBQ), and tetramethyl-p-benzoquinone (diquinone) to quench the excited states of chlorophyll (Chl) molecules in Photosystem I (PSI) was studied. Chl fluorescence emission measured with isolated PSI submembrane fractions was reduced following the addition of exogenous quinones. This quenching progressively increased with rising concentrations of the exogenous quinones according to the Stern–Volmer law. The values of Stern–Volmer quenching coefficients were found to be $3.28 \times 10^5$ M$^{-1}$ (DBMIB), $1.31 \times 10^4$ M$^{-1}$ (DCBQ), and $3.7 \times 10^3$ M$^{-1}$ (diquinone). The relative quenching capacities of the various exogenous quinones in PSI thus strictly coincided to those found for the quenching of Fo level of Chl fluorescence in isolated thylakoids, which is emitted largely by Photosystem II (PSII) [Biochim. Biophys. Acta (2003) 1604, 115–123]. Quenching of Chl excited states in PSI submembrane fractions by exogenous quinones slowed down the rate of P700, primary electron donor of PSI, photooxidation measured at limiting actinic light irradiances thus revealing a reduced photochemical capacity of absorbed quanta. The possible involvement of non-photochemical quenching of excited Chl states by oxidized phyloquinones, electron acceptors of PSI, and oxidized plastoquinones, mobile electron carriers between PSII and the cytochrome $b_6/f$ complex, into the control of photochemical activity of PSI is discussed.

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1. Introduction

The first step of energy transformation in photosynthesis is the conversion of a photon into an electronic excited state of a pigment molecule located in the antenna system. The excited state is transferred through the antenna to a reaction center as an exciton. The trapping of an exciton by a reaction center in either Photosystem I (PSI) or Photosystem II (PSII) induces a charge separation between the primary electron donor and an intermediate electron acceptor [1]. In isolated reaction center preparations, the quantum yield of primary charge separation is very close to unity [2]. However, each pigment of the antenna system has a certain probability of radiative emission and thermal dissipation. These processes decrease the quantum yield of primary charge separation. In intact photosynthetic tissues, special mechanisms are known to reversibly alter the probability of excitons to thermally dissipate in PSII antenna [3]. The above mechanisms are commonly referred to as non-photochemical quenching of chlorophyll (Chl) fluorescence, as that quenching of excited Chl molecules is not related to the photochemical trapping of excitons, but decreases the quantum yield of Chl fluorescence with a concomitant increase of heat emission [4,5].

“Energy-dependent” quenching related to the protonation of the peripheral light-harvesting complex of PSII, LHCII, [6] and state transition determined by the photophosphorylation of LHCCI and the following migration of phospho-LHCCI apart from PSII [7] constitute the major processes increasing heat dissipation of excitons in PSII. In addition to these...
mechanisms, static quenching of the Chl fluorescence yield by oxidized quinones modulates non-photochemically the photochemical capacity of PSII as well [8].

Unlike PSII, the information on the mechanisms of non-photochemical quenching of Chl excited states in PSI is restricted to the view that P700\(^{+}\), oxidized primary electron donor, acts as a very efficient non-photochemical quencher [9]. However, the acceptor A\(_1\) in PSI is a phylloquinone molecule [10,11] and PSI complexes contain two phylloquinone molecules per P700 [12,13]. Thus, similarly to PSII, quenching of excited states of Chl molecules by oxidized quinone derivatives cannot be ruled out in PSI. In addition, oxidized plastoquinones are likely to be in contact with PSI complexes as well as with PSII because of their random diffusion in photosynthetic membranes, despite the fact that they do not directly donate electrons to PSI [14].

One of the approaches often used in the studies of ‘‘plastoquinone’’-type non-photochemical quenching in PSI is the introduction of artificial substituted quinones into thylakoid membranes [15,16]. To evaluate whether excited states of Chl also respond to exogenous quinones in PSI, we examined the action of three substituted quinones [148], 2,5-dibromo-3-methyl-6-isopropyl-in PSI, we examined the action of three substituted quinones to quench Chl excited states of Chl also respond to exogenous quinones [8]. By oxidized quinones modulates non-photochemically the photochemical capacity of PSII as well [8].

Our work has revealed a high capacity of exogenous substituted quinones to quench Chl \(a\) fluorescence in PSI. A strict hierarchy in the exogenous quinones was observed according to their ability to decline the quantum yield of Chl fluorescence in isolated PSI submembrane fractions: DBMIB>DCBQ>duroquinone, which corresponds to that found in PSII [17]. DBMIB molecules introduced in PSI submembrane fractions also declined the rate of P700 photooxidation under limiting light, thus demonstrating a reduced photochemical capacity of PSI in the presence of oxidized quinones. These findings open the question of whether the redox state of plastoquinones, mobile electron carriers, can affect not only PSII, but also PSU photochemical capacity.

2. Materials and methods

2.1. Isolation of PSI submembrane fractions

PSI submembrane fractions were isolated from fresh spinach leaves obtained from a local market, according to the procedure of Peters et al. [18] with some modifications [19]. As reported earlier [20], the Chl \(a/b\) ratio in this preparation was above 6. Chl concentration and Chl \(a/b\) ratio were determined according to Porra et al. [21].

2.2. Preparation of duroquinol

Durohydroquinone was prepared from duroquinone as described previously [22]. About 2 mg of NaBH\(_4\) was added to 1 ml of ice-chilled stock solution of duroquinone prepared at a concentration of 25 mM in ethanol–ethylene glycol 1:1. After mixing, the solution was allowed to stand on ice for 5 min, during which time the reduction process was completed. There was enough moisture in the solvent to allow complete reduction of the quinone. Concentrated HCl (5 µl) was then added to the solution to stabilize the hydroquinone formed and to decompose the remaining NaBH\(_4\). No detectable re-oxidation of durohydroquinone occurred in this stock solution during 4–5 h experiments.

2.3. Fluorescence measurements

The room and low temperature (77 K) spectra of fluorescence emission were measured with a Perkin-Elmer LS55 spectrofluorimeter. Chl fluorescence was excited at 436 nm. The excitation and emission spectral widths were fixed at 5 and 2.5 nm, respectively. Equal Chl concentration (5 µg/ml, equivalent to 0.02 µM P700) was used for all the measurements.

2.4. Absorbance changes at 830 nm

Absorbance changes at 830 nm were assayed using a Pulse Amplitude Modulated (PAM) Chl fluorometer (Walz, Effeltrich, Germany) equipped with ED-P700DW dual-wavelength emitter–detector unit. White actinic light for measurements with the PAM device was obtained from a Fiber-Lite light source (Microview, Thornhill, ON, Canada) and controlled by an electronic shutter. The signals of absorbance changes were recorded using the Walz Data Acquisition System DA100. Chl concentration was 30 µg/ml during the measurements of absorbance changes, which were done in a cuvette with an optical path of 1.065 mm.

3. Results

The effect of three exogenous substituted quinones on photochemical properties of PSI was studied in isolated submembrane fractions. It should be noted that the PSI submembrane fractions used are obtained from a mild isolation procedure that retains all the components of PSI including the cytochrome \(b_6/f\) complex and plastocyanin [18] and they are very similar to PSI in whole thylakoid membranes. However, the preparation is devoid of PSII polypeptides as shown by Coomassie stained polyacrylamide electrophoresis gels [23]. It also does not evolve oxygen with DCBQ as electron acceptor and does not present light-induced variable Chl fluorescence (the authors observations).

Fig. 1 shows the room temperature Chl fluorescence emission spectra measured with isolated PSI submembrane
fractions in the absence of additive or in the presence of various concentrations of exogenous quinones. The spectra measured in untreated submembrane fractions showed a peak at 684 nm and a shoulder at 710 nm. The yield of Chl fluorescence declined following the increase in DBMIB concentration (Fig. 1A). It occurred equally at all wavelengths of the emission spectrum. Similarly to DBMIB, two other quinones were examined, DCBQ and duroquinone. They were found to quench Chl fluorescence as well, and the extent of quenching increased with concentration (Fig. 1B and C). Low temperature fluorescence emission spectra of the PSI submembrane fractions presented a maximum at 736 nm as previously reported [20]. This band was quenched if one of the above exogenous quinones was added to the PSI submembrane fractions prior to freezing of the sample, but the position of the emission peak was not affected (data not shown). However, the absorption spectrum of isolated PSI submembrane fractions was not influenced by the exogenous quinones.

In Fig. 2, the action of the reduced form of duroquinone ( durohydroquinone) on the fluorescence spectra of PSI submembrane fractions is presented. It is clear that no quenching of Chl fluorescence is observed with durohydroquinone. Thus we can conclude that the oxidized form of the quinones is required for the quenching.

Fig. 3 shows that the quenching of Chl fluorescence by substituted quinones occurred according to the Stern–Volmer law:

\[
\frac{F}{F'} = 1 + K_{SV}[Q],
\]

where \( F \) and \( F' \) are the Chl fluorescence emission integrated over wavelengths from 600 to 800 nm (see Fig. 1) 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 quencher. The slopes of linear fits, which characterize in Stern–Volmer plots of the relative efficiencies of various quenchers, were found to be highly different for the three exogenous quinones examined. Quinones were ranged according to the values of their Stern–Volmer quenching constants as DBMIB (\( K_{SV} = 3.28 \times 10^5 \text{ M}^{-1} \))–DCBQ (\( 1.31 \times 10^4 \text{ M}^{-1} \))–duroquinone (\( 3.7 \times 10^3 \text{ M}^{-1} \)).
Quenching of Chl excited states by exogenous quinones indicated that the photochemical processes in PSI could be restricted in favor of heat emission. To verify this hypothesis, we determined the rate of photooxidation of P700 under limiting light, i.e., under conditions where the photochemical capacity of PSI was restricted by the number of quanta absorbed by the antenna system and delivered to the primary donor to initiate charge separation in the special pair [P700 \text{A}_0] [24]. The expectation was that the rate of P700 photooxidation must be decreased by the addition of exogenous quinones capable of quenching the excited antenna Chls in the isolated PSI submembrane fractions. Fig. 4 presents the original traces of absorbance changes at 830 nm ($\Delta A_{830}$) induced by white light as low as 4.6 W m$^{-2}$ in isolated PSI submembrane fractions. Absorbance changes were measured either in the absence or in the presence of DBMIB given at various concentrations. All measurements were done in the presence of methyl viologen (3 mM) to prevent back electron transfer to P700$^+$ from reduced PSI acceptors. Positive absorbance changes initiated by the onset of actinic light indicated P700 photooxidation. Its rate progressively decreased with increasing concentrations of added DBMIB. Importantly, in contrast to the two other quinones used in this study, DBMIB did not affect the rate of the slowly proceeding dark P700$^+$ reduction, which was obtained from the relaxation of $\Delta A_{830}$ after cessation of actinic light irradiation (data not shown), and therefore, this quinone does not interfere directly with the photooxidation.

Fig. 5 illustrates the semi-logarithmic plots of the time courses of $\Delta A_{830}$ rise. The plots were fitted by a single first-order term thus revealing that the rate of P700 photooxidation was determined solely by actinic light irradiance. The slopes of the linear fits decreased as DBMIB concentration was raised. However, no deviation from the first-order kinetics was observed in the presence of DBMIB. That finding indicates that the addition of DBMIB mimics a reduction of actinic light irradiance, which slowed down $\Delta A_{830}$ rise as well (data not shown).

4. Discussion

In this study, we have shown that exogenous quinones in oxidized state are able to non-photochemically quench the excited states of Chl molecules in isolated PSI submembrane fractions. This quenching is described by the Stern–Volmer equation (Fig. 3), which coincides with the similar mode of action of artificial quinones on the Fo level of Chl fluorescence observed in whole thylakoid membranes [17,25]. Both PSII and PSI contribute to the Fo level measured in intact leaves or in isolated thylakoid membranes. However, the contribution of PSI to Fo, which varies...
among plant species, does not exceed 20–25% [26]. The above indicates that the previously reported non-photochemical quenching of Fo level of Chl fluorescence by exogenous quinones in thylakoid membranes [17] was caused preferentially by the action on PSIII emission. The quenching of Chl fluorescence emission in isolated PSI submembrane fractions by exogenous quinones represents, therefore, an independent process, whose features are, however, similar to Fo quenching in isolated thylakoids.

In the light-harvesting complex of PSI (LHCI) Chl molecules are associated with hydrophobic apoproteins with apparent molecular weights of 11–24 kDa that show some homology to LHCII, CP29 and CP24 Chl a/b-binding proteins [27,28]. That homology likely determines the similarity between the relative capacities of various exogenous quinones to quench Chl fluorescence in isolated PSI submembrane fractions (DBMIB>DCBQ>duroquinone) and in isolated thylakoids, in which they preferentially quenched Chl fluorescence emitted by PSII [17].

Quenching of excited states of Chl molecules by substituted exogenous quinones undoubtedly has a functional significance. This is evident from the reduced apparent rate of P700 photooxidation in isolated PSI submembrane fractions treated with DBMIB (see Figs. 4 and 5). Indeed, the apparent rate of P700 oxidation under continuous light reflects the balance between the true rate of its photooxidation and the rate of electron input from native or artificial donors (200 μM ascorbate in the case of isolated PSI submembrane fractions). As the rate of P700⁺ dark reduction was the same in the absence and in the presence of DBMIB, the decrease in the apparent rate of P700⁺ accumulation under light was due to the reduced photochemical capacity of PSI owing to a restricted exciton migration towards the reaction centers.

Thus, the quenching effect of quinones on excited states of Chl is obvious in PSI as well as in PSII. In PSII, oxidized plastoquinone molecules also quench the variable part of Chl fluorescence during the so-called thermal phase of the fluorescence induction [29]. It is still unclear, however, whether native quinones in PSI are capable of modulating the photochemical efficiency of PSI through a non-photochemical mode of action. As the phylloquinone acceptor A₁ is known to be reduced within 20–50 ps and to be re-oxidized within 0.1–0.2 μs by electron transfer to F₅ iron–sulfur cluster [30], rapidly proceeding changes in its redox state can barely influence non-photochemically the photochemical capacity of PSI under continuous irradiation. However, some continuous non-photochemical quenching of excitation in PSI related to A₁ molecules cannot be ruled out, as they are most of the time in oxidized state.

The A₁ molecule is tightly bound to the PSI core. In contrast, plastoquinone molecules deliver electrons from PSII to the cytochrome b₅/f complex, serving as mobile electron carriers [31]. Plastoquinone operates by diffusion through the membrane until it becomes bound to the Qₐ-binding site on the PSII complex (oxidized form) or to the Fe–S cluster on the cytochrome b₅/f complex (reduced form) [31]. The fast movement of plastoquinone molecules is restricted by local domains of membrane between PSII and the cytochrome b₅/f complex but slow long range diffusion is expected [32]. They can, therefore, be in a contact with the PSI complexes. Since only oxidized plastoquinone molecules are able to quench excited Chls [8,29], a non-photochemical action on PSI reactions would be expected to depend on the redox state of the plastoquinone pool.

Two types of PSI units were distinguished in the chloroplast, PSIIα located in the grana margins and PSIIβ located in the stroma lamellae [33]. The two types of PSI units are functionally related to different PSIII complexes, PSIIα and PSIIβ, respectively. Thus, plastoquinone molecules can be reduced by PSII and oxidized by the cytochrome b₅/f complex in both major domains of the membrane system of the chloroplast. The PSI submembrane fractions used in this study originated from stroma lamellae. Despite the differences between PSIIα and to PSIIβ found in both electron transfer processes and antenna size [33,34], it seems unlikely that PSI complexes from the grana margins would not respond to oxidized quinone molecules. This question must be, however, further clarified.

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References

[9] N.G. Bukhov, R. Carpenter, Measurements of photochemical quenching of absorbed quanta in Photosystem I of intact leaves using si-


