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Modified Photosystem II acceptor side properties upon replacement of the quinone at the Q_B site with 2,5-dimethyl-*p*-benzoquinone and phenyl-*p*-benzoquinone

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By using a simple heptane/isobutanol extraction procedure on aqueous suspensions of thylakoids, the secondary quinone acceptor molecule at the Q_B intermediate in Photosystem II was selectively removed and replaced with simple benzoquinone molecules, without significantly disturbing the primary quinone acceptor molecule at the Q_A intermediate or the oxidizing side of the Photosystem II reaction center. The functioning of these modified samples was monitored by thermoluminescence measurements and the following observations were made. The extracted thylakoids, without any additions, exhibited a flash-induced thermoluminescence band identical with a DCMU-generated band (arising from $S_2Q_A^-$ charge recombination) rather than a normal B band (arising from $S_2Q_B^-$ charge recombination). The band did not display period four behavior in intensity following excitation by a sequence of light flashes. Upon the addition of either 2,5-dimethyl-*p*-benzoquinone or phenyl-*p*-benzoquinone to the extracted thylakoids, new thermoluminescence bands were generated. These new bands exhibited peak-temperature positions, intensities and room temperature decay kinetics that were distinctly different from each other as well as from the DCMU-generated band and the normal B band. However, the benzoquinone-generated bands remained sensitive to DCMU addition and did display period four behavior in intensity in a sequence of light flashes. These observations, therefore, indicate that the functioning of the Q_B intermediate in Photosystem II can be reactivated in the extracted thylakoids by the addition of simple benzoquinones, although with modified properties. For comparison with the above results, data are also reported on heptane/isobutanol-extracted lyophilized thylakoids which were subsequently reconstituted with native plastoquinone.

Introduction

In the photosynthetic transfer of electrons from water to $NADP^+$, electron flow is mediated by a 'two-electron gate' intermediate, Q_B. The Q_B intermediate links the one-electron carriers (pheophytin and Q_A) on the immediate reducing side of the Photosystem II (PS II) reaction center chlorophyll *a* molecule (P-680) with the two-electron carriers of the intersystem plastoquinone (PQ) pool [1,2]. It is most likely located on a polypeptide subunit (M_r = 32–33 kDa) of the PS II chlorophyll-protein complex and contains a bound quinone molecule which, upon a single reduction step, can form a stable semiquinone anion (Q_B^-) (for reviews, see Refs. 3 and 4). According to current concepts, the Q_B quinone is defined as a

Abbreviations: DCMU, 3-(3,4-dichloro)-1,1-dimethylurea; DMBQ, 2,5-dimethyl-*p*-benzoquinone; PBQ, phenyl-*p*-benzoquinone; PQ, plastoquinone; Q_A, primary quinone acceptor, Q_B, secondary quinone acceptor; PS II, Photosystem II; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Chl, chlorophyll.

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bound PQ molecule which readily exchanges with the intersystem PQ pool in its fully oxidized and fully reduced states, but not in its semiquinone form [5,6]. The structural and chemical properties of the Q_B quinone-binding site which confer stability to the semiquinone form, however, are not known.

Concurrently it has been indicated in many studies that simple benzoquinone molecules (e.g., *p*-benzoquinone and dibromothymoquinone, see Refs. 7 and 8) and commonly used herbicide molecules (e.g., DCMU and atrazine, see Refs. 9–11) compete with PQ binding to the Q_B intermediate. However, it is not yet clear whether all simple benzoquinone and herbicide molecules bind directly at the Q_B binding site or at another site on the protein [12,13]. All simple benzoquinone and herbicide binding studies were made on samples containing the functional PQ.

To gain additional information about the structural and chemical properties necessary for the functioning of the Q_B intermediate, it would be advantageous to remove completely the native bound quinone at the Q_B binding site. In the standard procedure to remove quinones from photosynthetic membranes, lyophilized material is typically extracted with organic solvents. However, this procedure removes not only the PQ pool and the quinone at the Q_B binding site, but also the quinone at the Q_A binding site [14] and possibly a bound quinone which may function on the oxidizing side of PS II as well [15,16]. By contrst, in a preliminary report [17], we showed that by using a simple heptane/isobutanol extraction procedure on aqueous suspensions of thylakoids, the functional PQ pool can be removed and the Q_B intermediate inactivated, without significantly disturbing the Q_A intermediate or the oxidizing side of PS II. In this study, the functional transfer of electrons to the Q_A and Q_B intermediates was monitored by the flash-induced thermoluminescence emission bands which arise from the charge recombination reactions between the semiquinone anions formed on the PS II acceptor side and the S_2 and S_3 charge states of the water-splitting complex (for review, see Refs. 18 and 19). After extraction, all normal thermolumenscence bands from $S_2Q_B^-$ and $S_3Q_B^-$ charge recombination were lost while the thermoluminescence band from $S_2Q_A^-$ charge recombination appeared. Upon the subsequent addition of simple benzoquinone molecules, variously modified thermoluminescence bands could be generated, indicating that quinone binding to the PS II acceptor side could be restored under these conditions.

In this communication, we extend our initial observations to an analysis of the thermoluminescence properties of heptane/isobutanol-extracted thylakoids specifically reconstituted with 2,5-dimethyl-*p*-benzoquinone (DMBQ) and phenyl-*p*-benzoquinone (PBQ). The results indicate that the functioning of the Q_B intermediate can be reactivated in these samples, although with modified properties, by the simple benzoquinone addition. To compare results, we also report our findings after heptane/isobutanol extraction of lyophilized thylakoids which were subsequently reconstituted with native PQ.

Experimental procedures

Thylakoids were prepared from market spinach according to standard procedures [20] and stored with 30% glycerol at $-80\,^{\circ}C$ until used. For the extraction of aqueous samples, thylakoids were suspended at 300 μg Chl/ml in a buffer medium consisting of 20 mM Hepes (pH 7.5), 400 mM sucrose, 10 mM NaCl, 30% (v/v) glycerol and 3% (v/v) isobutanol. Three parts heptane were added to one part of the thylakoid suspension (usually 9 ml of heptane was added to 3 ml of the suspension) in a 50 ml capped tube. The tube was positioned horizontally onto a rotary shaker and shaken vigorously for 45 min (unless otherwise noted in the text) at room temperature (18–20 °C) in the dark. After extraction, the pale yellow organic phase was removed and the aqueous sample suspension was illuminated for 1 min in continuous white light and then allowed to dark adapt for 7–10 min at room temperature before being stored on ice. The preillumination treatment was found to be necessary to release the remaining tightly bound (semi)quinone at the Q_B binding site (see Ref. 17). Freshly extracted aqueous thylakoid samples were used in all experiments.

For the extraction of lyophilized samples, thylakoids were first freeze-dried in a round bottom flask. Heptane was added to the dried

thylakoids roughly in the same proportion as used above (i.e., 3 ml heptane per 300 μg Chl). Isobutanol was subsequently added to 3% (v/v) of the heptane total volume and the flask was then gently swirled for 45 min at room temperature in the dark. The deep yellow organic phase was decanted off and residual solvent removed by air drying. Finally, the extracted-lyophilized thylakoids were suspended to 300 μg Chl/ml in the buffer medium defined above using a glass homogenizer. After lyophilization, however, it was difficult to obtain a fine suspension comparable to the original sample.

For quinone reconstitution, DMBQ and PBQ were simply added to the extracted aqueous thylakoid suspensions to the concentrations indicated in the text from concentrated stock solutions in 100% ethanol (final ethanol concentrations in the samples were less than 3%). For PQ reconstitution, purified PQ (1 mg per mg Chl) was added to a few ml of heptane containing the unsuspended, extracted-lyophilized thylakoids and allowed to dry slowly under a filtered N_2 gas stream. The dried, reconstituted lyophilized thylakoids were then suspended in the buffer medium as described above. DMBQ and PBQ were obtained from Sigma Chemical Company. Purified PQ was kindly prepared by H. Koike from spinach basically according to the procedure given in Ref. 21.

Thermoluminescence measurements of the samples adsorbed onto small filter paper segments (2 × 2 cm) were performed as described in Ref. 22, but with an instrument having an improved resolution. Sample suspensions at 300 μg Chl/ml were first illuminated with continuous white light (0.7 mW/cm^2) and allowed to dark-adapt at room temperature for 7 min to ensure a reproducible initial S-state distribution. However, it was found that excessive illumination of extracted samples containing the simple benzoquinones would tend to reduce the subsequent effects on the thermoluminescence, probably due to incomplete reoxidation of the benzoquinols during the ensuing dark-adaptation period. Consequently, optimal illumination periods had to be empirically determined for each sample. After the dark-adaptation period, 80 μl of a sample suspension was applied to the filter paper segment. Except for the experiments reported in Figs. 3 (bottom) and 4, the samples on the filter paper segments were cooled to 2°C, flash-illuminated with one or a series of xenon light flashes (4 μs, 2 J) provided by a Sugawara MS-230 stroboscope, and then quickly frozen in liquid N_2. In those experiments employing multiple flash excitation, the repetition frequency was 1 Hz. For the experiments in Figs. 3 (bottom) and 4, the samples on the filter paper segments were kept at room temperature, given one flash and then allowed to remain in the dark at room temperature for the various times indicated before being quickly frozen in liquid N_2. The thermoluminescence was recorded as a function of the sample temperature during heating at a rate of 0.6–0.8°C/s.

Results

Quinone reconstitution in extracted samples

In uninhibited thylakoids, after a single light flash excitation, a major thermoluminescence emission band is induced, having a peak-temperature position at about 35–40°C under the experimental protocol that we employ. This so-called 'B band' has been clearly analyzed in terms of $S_2Q_B^-$ charge recombination. After addition of the inhibitor DCMU to the sample, to block electron flow at Q_B, the flash-induced thermoluminescence emission band shifts to a lower peak-temperature position, at about 5–10°C. Under this condition, the so-called 'D band' was shown to arise from $S_2Q_A^-$ charge recombination. Thus, thermoluminescence measurements provide a direct monitor of the S-states as well as the acceptor side of PS II [22,23].

As initially reported in Ref. 17, after extraction of aqueous suspensions of thylakoids with a heptane/isobutanol mixture (which are labeled as HIE in the figures), the observed thermoluminescence emission band appears as a D band, in both intensity and peak-temperature position. Fig. 1 shows a plot of the thermoluminescence peak-temperature position as a function of extraction time. An extraction time of at least 30 min is required to generate a D -band-like thermoluminescence emission. To ensure a complete extraction effect, a 45 min extraction time was used in all subsequent measurements. Fig. 1 also shows that upon the addition of DCMU to samples exhibiting the

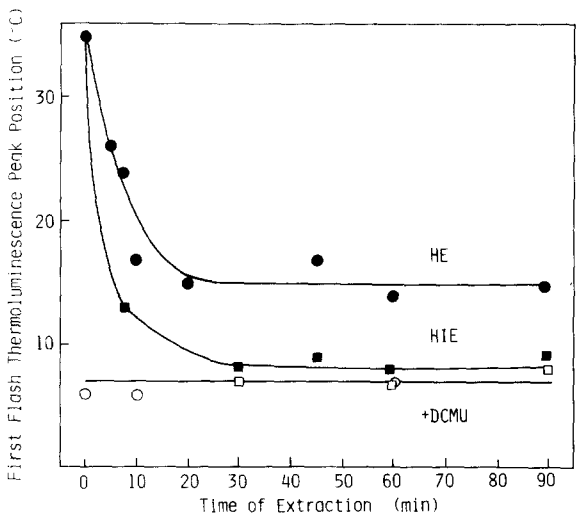

Fig. 1. Plots of the flash-induced thermoluminescence peak-temperature position as a function of heptane (HE, ●) or heptane/isobutanol (HIE, ■) extraction time on aqueous suspensions of thylakoids. Closed symbols, without DCMU. Open symbols, with 5 μM DCMU.

complete extraction effect, no further change in the thermoluminescence peak-temperature position is observed. These results, therefore, indicate that Q_B^- is no longer available for charge recombination while the Q_A intermediate remains relatively unperturbed by this extraction procedure.

That the inactivation of the Q_B intermediate by the above extraction procedure requires isobutanol is also indicated in Fig. 1. Extraction of aqueous thylakoid suspensions with heptane alone (which are labeled as HE in the figure) results in an observed thermoluminescence band that is intermediate between the normal D and B bands (i.e., with a peak-temperature position at about 15°C), even after a 90 min extraction time. As also shown in Fig. 1, this thermoluminescence band is DCMU-sensitive. Interestingly, however, only about 70% of the functional PQ pool is removed under this condition [17].

Upon the addition of a variety of simple benzoquinones to the heptane/isobutanol-extracted aqueous suspensions of thylakoids, variously modified thermoluminescence emission bands are generated [17]. Fig. 2 shows the thermoluminescence measured in such extracted thylakoids with increasing concentrations of DMBQ and PBQ. We chose these two benzoquinones as representative

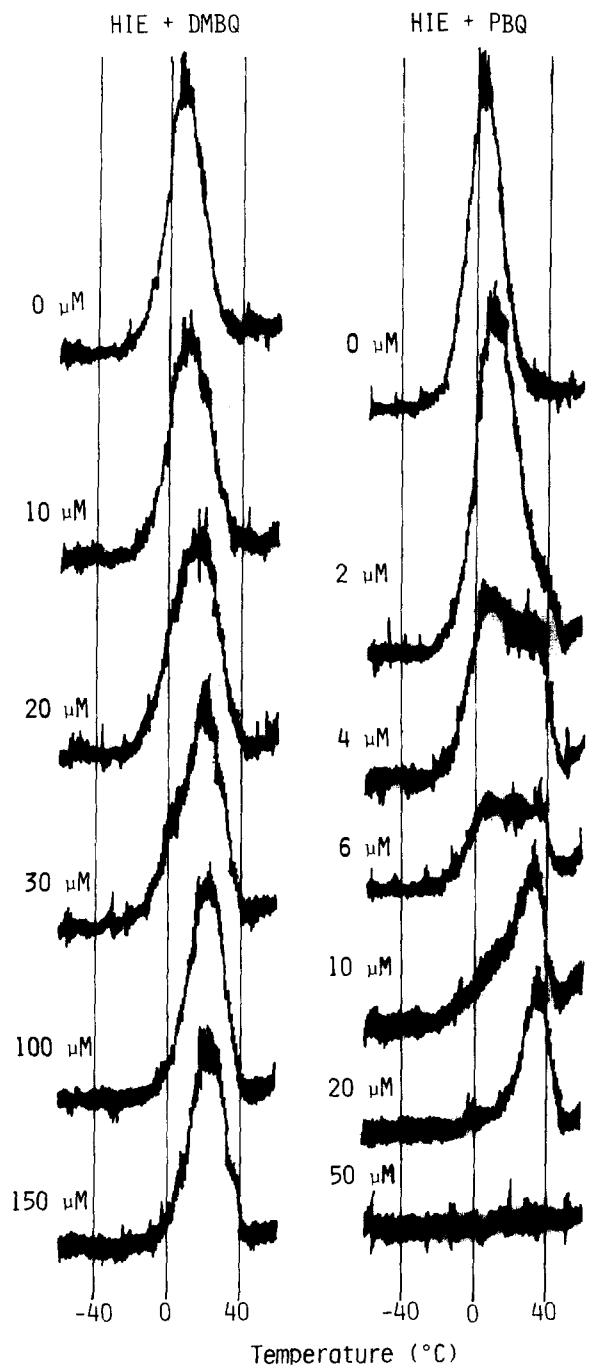

Fig. 2. Flash-induced thermoluminescence glow curves of heptane/isobutanol-extracted aqueous suspensions of thylakoids (HIE) upon the addition of increasing concentrations of either DMBQ or PBQ. The thermoluminescence intensity is in arbitrary units, although the same amplifier gain was used in all measurements.

of the effects for two reasons. First, these benzoquinones completely convert the inherent thermoluminescence band of the extracted samples to a modified thermoluminescence band at concentrations which do not overly quench the thermoluminescence yield (in general, the presence of high concentrations of electron acceptors will quench the thermoluminescence). Second, DMBQ and PBQ generated two very distinct thermoluminescence bands. As shown in Fig. 2, DMBQ generates a thermoluminescence band intermediate between the normal D and B bands, with a peak-temperature position at about 22°C, while PBQ generates a thermoluminescence band with a peak-temperature position near what is observed for the normal B band, at about 38°C. It is apparent from Fig. 2 that intermediate concentrations of the benzoquinones generate a mixture of the inherent and modified thermoluminescence bands. One interesting feature of the PBQ-generated band is that its intensity is significantly lower than the other thermoluminescence bands. This reduced intensity is more than can be accounted by the general quenching effect; for, as shown in Ref. 17, upon the addition of DCMU to PBQ-treated extracted samples, a D band is generated which is twice as intensive. Usually, DCMU does not cause such large changes in the thermoluminescence intensity [22,23].

To compare results and also since it is extremely difficult to reconstitute native PQ into aqueous thylakoid suspensions, we also performed the heptane/isobutnol extraction procedure on lyophilized thylakoids (which are labeled as lyophilized HIE in the figure). The results are shown in Fig. 3 (top). The extracted lyophilized samples exhibit a complete loss of thermoluminescence in the temperature range usually ascribed to the normal D and B bands, which is consistent with the loss of the Q_B quinone, the Q_A quinone and/or the possible oxidizing side quinone by this procedure. Upon reconstitution of these samples with PQ, a broad thermoluminescence emission band is restored, with a peak-temperature position near the expected B band position. However, this reconstituted band is apparently multicomponent. Fig. 3 (bottom) shows a plot of the peak-temperature position as a function of the dark time at room temperature after a single flash excitation.

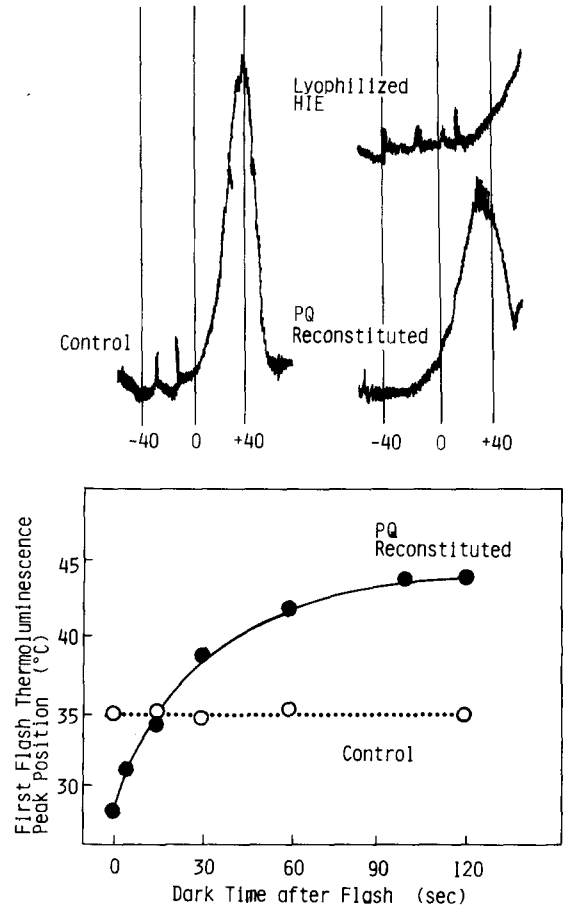

Fig. 3. Top, Flash-induced thermoluminescence glow curves for a control thylakoid sample, a heptane/isobutnol-extracted lyophilized thylakoid sample resuspended in aqueous medium (Lyophilized HIE), and an extracted lyophilized thylakoid sample reconstituted with plastoquinone-A and resuspended in aqueous medium (PQ Reconstituted). Bottom, Plot of the thermoluminescence peak-temperature position as a function of dark time at room temperature after a single excitation light flash for a control thylakoid sample (○) and a heptane/isobutanol-extracted lyophilized thylakoid sample reconstituted with plastoquinone-A and resuspended in aqueous medium (●).

In contrast to the control B band, the PQ reconstituted band shifts to significantly higher peak-temperature positions with increasing time. This behavior is characteristic of a multicomponent band, where the loss of more rapidly decaying, lower-temperature emission components causes a shift in the observed thermoluminescence peak-temperature position towards the more slowly decaying, higher-temperature emission components

with time after excitation. Most likely the lyophilization procedure damages the quinone-binding sites.

Fig. 4 shows a logarithmic plot of the flash-induced thermoluminescence band intensity for the various samples as a function of dark time at room temperature after the flash. As reported previously [22,23], the normal B band of the control displays an exponential decay with a half time of about 45 s, which is consistent with the half-lifetime of the S_2 state in thylakoids. Upon the addition of DCMU, the decay in the thermoluminescence intensity is much more rapid, with a half time of about 3 s. In this case the deactivation is caused by the reoxidation of Q_A^-. For the heptane/isobutanol-extracted aqueous suspensions of thylakoids, the thermoluminescence decay almost parallels what is observed for the control sample containing DCMU. This result provides further evidence that Q_B^- no longer functions in this sample and that Q_A remains intact. The

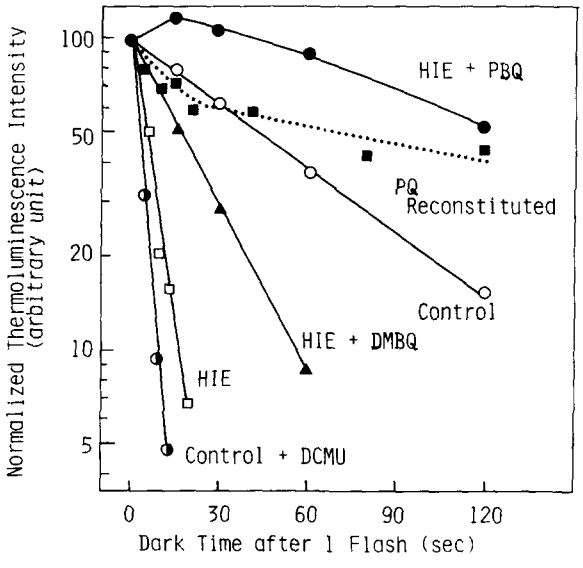

Fig. 4. Plots of the thermoluminescence intensity (normalized to the same zero time value) as a function of dark time at room temperatures after a single excitation light flash for control thylakoids without any additions (○) and with 5 μM DCMU (○); heptane/isobutanol-extracted aqueous suspensions of thylakoids without any additions (HIE, □), with 50 μM DMBQ (▲) or with 20 μM PBQ (●); and heptane/isobutanol-extracted lyophilized thylakoids reconstituted with plastoquinone-A and resuspended in aqueous medium (■).

DMBQ-generated thermoluminescence band also exhibits a single exponential decay, but with a half time of about 30 s. This result is consistent with the location of the peak-temperature position of this band as being intermediate between the D and B bands. In contrast, the PBQ-generated thermoluminescence band exhibits a rather peculiar kinetics. Initially, in about the first 20 s after the flash, there is no change or a small increase in the thermoluminescence intensity, which is then followed by a slow decay with an estimated half time of about 90 s. The small initial increase or lack of change in the thermoluminescence decay course would formally indicate the formation of a new thermoluminescence species. Finally, for the PQ reconstituted lyophilized thylakoids, the thermoluminescence decay is complex, apparently with several exponential components. This result would be consistent with the apparent multicomponent composition of the observed thermoluminescence band (Fig. 3).

Restoration of period four behavior in reconstituted samples

When the intensity of the B band for a control sample is measured after each flash in a sequence of light flashes, a periodicity of four is observed [24]. The thermoluminescence intensity is determined by the relative distribution of the S_2 and S_3 states according to the Kok model [25,26], the relative contribution of these S-states to the thermoluminescence yield (it has been estimated that the thermoluminescence yield arising from $S_2Q_B^-$ is about one half of that arising from $S_3Q_B^-$ [27]) and the initial concentration of Q_B^- [27]. For thylakoids which are thoroughly dark-adapted (6 h in the dark on ice after a 1 min illumination period), the S-states deactivate to their usual dark distribution while the Q_B/Q_B^- ratio adjusts to a relatively high value (estimated to be about $Q_B/Q_B^- = 70:30$ [27]). In this situation, in a sequence of flashes the thermoluminescence intensity displays peaks after the first and fifth flashes. On the other hand, for thylakoid samples given only a short dark-adaptation period (7 min in the dark at room temperature after a 1 min illumination period), the S-states deactivate to the same dark distribution as in the above case, but the Q_B/Q_B^- ratio adjusts to a lower value (estmated to

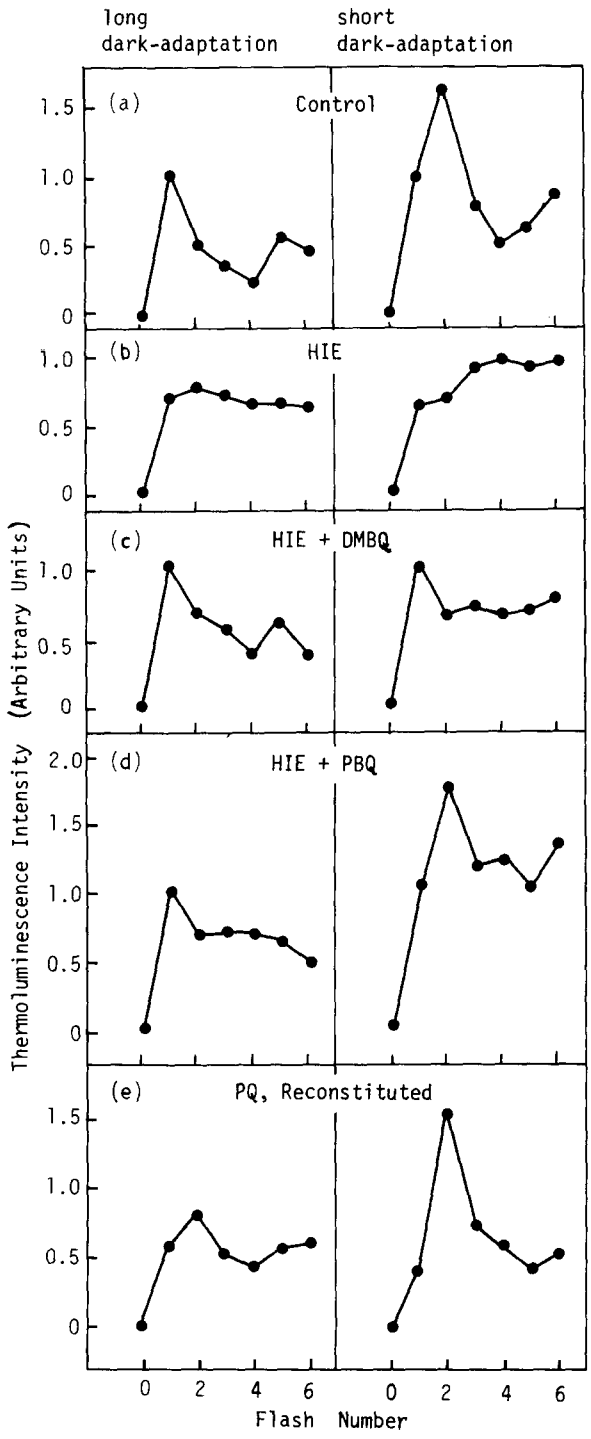

Fig. 5. Plots of the thermoluminescence intensity measured as a function of flash excitation number. (a) Control thylakoids; (b) heptane/isobutanol-extracted aqueous suspensions of thylakoids (HIE) (c), (d) heptane/isobutanol-extracted aqueous suspensions of thylakoids containing either 50 μM DMBQ (c) or 20 μM (d); (e) heptane/isobutanol-extracted lyophilized thylakoids reconstituted with plastoquinone-A and resuspended in aqueous medium. The samples were subjected either to long dark-adaptation conditions (6 h on ice in the dark after a 1 min illumination period) or short dark-adaptation conditions (7 min at room temperatures in the dark after a 1 min illumination period).

be about $Q_B/Q_B^- = 50:50$ [27]), since Q_B^- is a relatively long-lived species over this dark time. In this situation, the thermoluminescence intensity displays peaks after the second and sixth flashes. As shown in Fig. 5a, our control thylakoid samples follow the above flash patterns under the two dark-adaptation conditions.

When the heptane/isobutanol-extracted aqueous suspensions of thylakoids are subjected to the same dark-adaptation conditions described above, no periodicity of four is observed in the thermoluminescence intensity in a sequence of light flashes in either case (Fig. 5b). These samples behave as control samples containing DCMU. The periodicity of four is lost because the Q_B intermediate is no longer capable of shuttling electrons from Q_A^- to the intersystem electron carriers.

Upon reconstitution of the extracted thylakoids with DMBQ, under the long dark-adaptation condition, the basic periodicity of four is restored in the thermoluminescence intensity, with the first peak occurring after the first flash (Fig. 5c). Under the short dark-adaptation condition, however, the period four behavior intensity is also implicated, but the first peak still occurs after the first flash and not after the second flash as in the control. This result may indicate that under this condition, the semiquinone form which is involved in the charge recombination process may not be as dark-stable as it is in control samples. Upon reconstitution of the extracted thylakoids with PBQ, the basic control patterns in the thermoluminescence are restored under the two dark-adaptation conditions (Fig. 5d). Likewise, for the PQ reconstituted lyophilized thylakoids, the basic control patterns are also observed (Fig. 5e), although under the long dark-adaptation condition the pattern is not as clear cut as it might be. In this case, deviation from the control pattern may be a consequence of the damage induced by the lyophilization procedure and could formally indicate that

Q_B^- is even more dark stable than normal or that the S-states have been altered.

Since it was shown that addition of ferricyanide to heptane/isobutanol-extracted aqueous suspensions of thylakoids could restore the normal O_2 flash yield pattern to about 75% of the control level with no influence on the thermoluminescence properties [17], damage to the oxidizing side of PS II by this extraction procedure appears to be minimal. Thus, the above results indicate that the functioning of the Q_B intermediate can be restored in these extracted samples by the addition of simple benzoquinone molecules.

Discussion

It is clear from the results reported in this communication that the heptane/isobutanol-extracted aqueous suspensions of thylakoids behave in the same manner as control thylakoids containing DCMU, at least in terms of the thermoluminescence properties (Figs. 1, 4, 5; Ref. 17). Upon the addition of DCMU to these extracted samples, no further changes in the thermoluminescence peak temperature position or intensity are observed (Fig. 1; Ref. 17). Thus, it is likely that the quinone at the Q_A intermediate remains relatively undisturbed while the quinone at the Q_B intermediate is removed by this extraction procedure.

Unlike DCMU-treated samples, however, the addition of simple benzoquinone molecules to the extracted samples will generate a range of modified thermoluminescence bands, depending upon the benzoquinone used [17]. For the two benzoquinones employed in this study, DMBQ and PBQ, the thermoluminescence bands that are generated are quite distinct in terms of the peak-temperature position (Fig. 2) and the room temperature decay kinetics after a single flash excitation (Fig. 4), both from each other and from the DCMU-generated D band and the normal B band of the control. The benzoquinone-generated bands in these samples obviously reflect benzoquinone binding to the acceptor side of PS II, which can subsequently take part in the charge recombination reactions that give rise to thermoluminescence. However, it will require further investigations to evaluate how the altered thermoluminescence properties in these samples are related to the structural and chemical properties of the individual benzoquinones and their binding site(s) in PS II.

Nevertheless, of particular interest are the observations that after the addition of either DMBQ or PBQ to the heptane/isobutanol-extracted aqueous suspensions of thylakoids, the period-four behavior in a sequence of light flashes is restored in the thermoluminescence intensity (Fig. 5) as well as in the O_2 flash yields [17]. Although the contribution of non-B-type PS II centers to the thermoluminescence is not known (as much as 50% of the centers may be non-B-type in higher plants [28,29]), kinetic analysis of the thermoluminescence intensity variations indicates that both the cycling of the S-states and the two-electron gate operation at the Q_B intermediate govern the period four behavior [19,27]. Since it was found that the addition of ferricyanide to these extracted samples could restore the O_2 flash yield oscillations but had no consequent effect on the inherent thermoluminescence properties, it appears that the oxidizing side of PS II and the cycling of the S-states are little affected. (Apparently, ferricyanide is capable to accept electrons from PS II prior to the Q_B intermediate under these conditions.) Assuming this to be the case, the restoration of the period four behavior in the intensities of the modified thermoluminescence bands generated by DMBQ and PBQ would, therefore, indicate that the benzoquinones do indeed bind at the Q_B intermediate and reactivate the two-electron gate operation.

A recent report [30] has also implied that high-potential benzoquinones, such as DMBQ and PBQ, are able to occupy the Q_B site in PS II, even in the presence of the native PQ. In this study, however, it was suggested that the bound ferrous iron associated with the electron acceptor side of PS II (which is now correlated with the Q_{400} acceptor [31]) becomes oxidized upon photoreduction of bound benzoquinones to the semiquinone forms. This so-called photoreductant-induced oxidation of the bound ferrous iron by high-potential benzoquinones would appear at first to be inconsistent with the ability of DMBQ and PBQ to generate thermoluminescence bands, which inherently requires the participation of bound semiquinone

forms. But the two sets of observations may be reconciled by considering the photoreductant-induced oxidation to be an equilibrium condition, i.e.,

$$Fe^{2+}Acc \xrightarrow{h\nu} Fe^{2+}Acc^{-} \overset{2H^+}{\rightleftharpoons} Fe^{3+}AccH_2$$

where Acc represents the bound benzoquinone molecule. Thus, under the conditions used in Ref. 30, the above equilibrium would presumably be shifted to the right, whereas under the conditions of the present work the equilibrium would presumably be shifted to the left. It is interesting to point out that for PBQ, which was the most efficient benzoquinone used to catalyze the bound ferrous iron oxidation [30], the observed thermoluminescence band exhibits a much lower intensity than might be expected (Fig. 2). Further work will be required to determine how the bound ferrous iron behaves in the extracted thylakoids and how benzoquinone/benzoquinol concentrations may affect the above proposed equilibrium condition.

For comparison with the above results, we also report the data on heptane/isobutnol-extracted lyophilized thylakoids. In these samples, all thermoluminescence is lost in the usual temperature range ascribed to $S_2Q_A^-$ and $S_2Q_B^-$ charge recombination (Fig. 3, top). This loss of the thermoluminescence would indicate that, in addition to the Q_B quinone, the Q_A quinone and/or the possible quinone located on the oxidizing side of PS II are removed by this extraction procedure as well. However, in contrast to the heptane/isobutanol-extracted aqueous suspensions of thylakoids described above, addition of simple benzoquinone molecules could not restore thermoluminescence in the usual temperature range (data not shown). Restoration of the thermoluminescence could be achieved, however, after reconstitution of these samples with the native PQ (Fig. 3, top). The restored thermoluminescence band in this case appears as a multicomponent band under conditions in which a single band is expected (Fig. 3, bottom). This probably indicates that the quinone-binding sites are damaged by the lyophilization procedure. Indeed, we observed that at least 50%, and typically more, of the O_2 activity is lost after the lyophilization procedure only, without extraction (data not shown).

In conclusion, we have shown in this communication that a simple heptane/isobutanol extraction procedure on aqueous suspensions of thylakoids can be used to remove specifically the functional quinone at the Q_B intermediate in PS II. Upon reconstitution of these extracted samples with the simple benzoquinone molecules DMBQ and PBQ, the functioning of the PS II acceptor side was restored, although with altered properties. We feel that this experimental approach will be useful in future comparative quinone replacement studies on the PS II acceptor side.

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