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Interaction of exogenous quinones with photosystem II in inside-out thylakoids

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The presence of exogenous quinones PPBQ, DCBQ and DMQ in inside-out thylakoids alters the oscillation pattern with period 4 of the flash-induced fluorescence yield. The results can be interpreted by assuming that at ambient temperature PPBQ replaces PQ in 100% of the PS II centers detected by fluorescence, DMQ and DCBQ being bound to 40–60% of these centers only. The evaluation of the percentage of double hits in a 3 μs flash allows estimation of the electron-transfer rate from Q_A^- to Fe^{3+}, which varies from 9 μs (PPBQ) to 20 μs (DMQ).

Oxygen evolution; Photosystem II; Chlorophyll fluorescence; Quinone; (Inside-out thylakoid)

1. INTRODUCTION

PS II couples light-induced charge separation with the reduction of plastoquinone and the oxidation of water. Of the seven polypeptides that comprise the minimal unit of PS II, attention has been focused on two of these, D_1 and D_2, as binding key elements of the photochemical system [1]. In polypeptide D_1, in a special binding niche (Q_B site), Q_B links the one-electron carriers (pheophytin and Q_A) with the two-electron carriers of the intersystem plastoquinone pool [2,3]. Q_B is a bound quinone molecule which, upon a single reduction step, can form a stable semiquinone anion (Q_B^-). Once Q_B^- has been fully reduced to the quinol form, it readily exchanges with the intersystem oxidized PQ pool [4].

Exogenous quinone acceptors are often added to PS II-enriched membranes in order to diminish limiting reactions on the acceptor side. However, there is somewhat contradictory evidence concerning the efficiency of these quinones to compete with PQ for binding to the Q_B site [5–7]. By EPR observations, exogenous quinones have been shown to induce the oxidation of Fe^{2+}, situated between Q_A and Q_B [8,9]. In a sequence of saturating laser flashes (15 ns pulse), alternating oxidation of Fe^{2+} on odd-numbered flashes is followed by photoreduction of Fe^{3+} on even-numbered flashes in the presence of PPBQ [8]. Zimmermann and Rutherford [8] have explained their results by the following reactions: after the first flash of the series, Q_A^- is reoxidized by PPBQ. The semiquinone form of PPBQ thus produced is unstable (in contrast to Q_B^- which is tightly bound in the Q_B site) and oxidizes Fe^{2+} to Fe^{3+} to form a stable quinol. At the following flash, Q_A^- is reoxidized by reducing Fe^{3+} to Fe^{2+}. A similar interpretation has been made by Petrouleas and Diner who observed iron oxidation in PPBQ-treated samples which had been thawed after low-temperature illumination [9].

By comparing the flash-induced oscillations with period 4 of the oxygen and fluorescence yield, it has been shown that two types of oxygen-evolving centers are indeed functionally distinct [10,11], and that the oxygen-evolving centers responsible

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Abbreviations: PS, photosystem; PPBQ, phenyl-*p*-benzoquinone; DCBQ, 2,5-dichloro-*p*-benzoquinone; DMQ, 2,5-dimethylbenzoquinone; PQ, plastoquinone; Q_A (Q_B), primary (secondary) quinone acceptor of PS II

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for the fluorescence oscillations are connected to Q_B and the plastoquinone pool [11]. Therefore, the interaction of exogenous quinones with the Q_B site can be investigated by analysing the effects of PPBQ, DCBQ or DMQ on the flash-induced fluorescence yield.

2. MATERIALS AND METHODS

Inside-out thylakoids were obtained by mechanical disintegration of pea chloroplast thylakoids, followed by phase partitioning according to Åkerlung and Andersson [12]. The suspension used was a medium containing 300 mM sorbitol, 10 mM NaCl, 5 mM $MgCl_2$, 40 mM Mes-NaOH buffered at pH 6.5.

Flash excitation was provided by a Stroboslave General Radio flashlamp (3 μs at half-peak height). All flashes in the experiments were saturating. In kinetic measurements (microsecond range), flashes were filtered using a Schott BG 38.

Fluorescence experiments were performed using the apparatus described in [11]. Flashes and the measuring light (12 ms in most experiments) were triggered by a microcomputer (Apple II plus). After each flash of the same series, the chlorophyll *a* fluorescence signal was amplified, recorded in the transient waveform recorder and partially stored in the microcomputer through a fast parallel interface. Records of the fluorescence decays were obtained following a variable time interval after all the flashes of a sequence, as well as the printing of the fluorescence yield measurements F_v and F_0. F_0, the fluorescence yield after darkness, was then subtracted from F_v, the fluorescence yield after each flash.

The fluorescence yield patterns induced by the first 16 flashes after dark adaptation were fitted to values of the fluorescence increase calculated from a model using varying values for misses α (centers not converted on a flash, but may become so on a subsequent flash), double hits β (two conversions on a flash) and percentage of centers remaining active after each flash of a sequence, z [11]. Consequently, $1 - z$ represents the percentage of active centers lost after each flash of a sequence and which remain non-functional during the entire sequence ($z = 1$, when the number of active centers is constant as a function of flash number).

3. RESULTS AND DISCUSSION

Fig.1 shows the fluorescence yields, 73–85 ms after each flash of a series of 16 flashes (only 11 shown) in dark-adapted inside-out thylakoids, in the presence of 0.5 mM ferricyanide and various exogenous quinones. In the presence of ferricyanide (fig.1a), the period 4 fluorescence oscillations appear to be superposed on a constant fluorescence level, which corresponds to the proportion of Q_A^- not yet reoxidized 85 ms after each flash of the series. The effects of PPBQ, DCBQ or DMQ on PS II were studied in the presence of ferricyanide in order to allow reoxidation of these quinones between flashes after their reduction by electrons coming from the acceptor side of PS II.

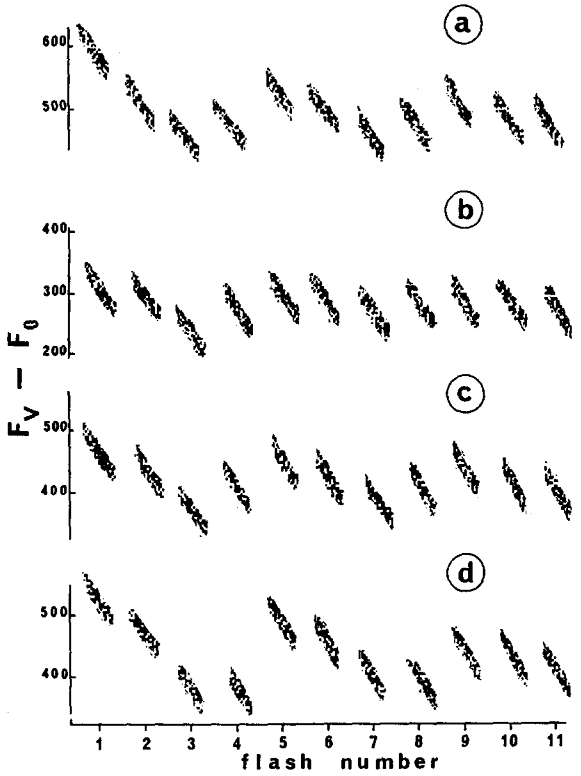

Fig.1. Fluorescence yield decays (73–85 ms) induced by each of the first 11 flashes of a series of flashes in inside-out thylakoids at pH 6.5, in the presence of: (a) 0.5 mM ferricyanide; (b) 0.5 mM ferricyanide and 10 μM PPBQ; (c) 0.5 mM ferricyanide and 10 μM DCBQ; (d) 0.5 mM ferricyanide and 50 μM DMQ. Samples were dark-adapted for 10 min except in (a) where this period was 1 h. Flash interval was 730 ms.

In fig.2 and table 1, the least-squares fitting method applied to the experimental patterns of fluorescence yield measured 80 ms after each flash of a series reveals the presence of very few misses. The miss percentage α varied from 0 to 5% in all samples studied. A very low value of α was previously determined in the PS II centers responsible for the fluorescence oscillations [10,11]. This value increased only slightly in the presence of PPBQ. The main changes induced by the presence of exogenous quinones are of two kinds.

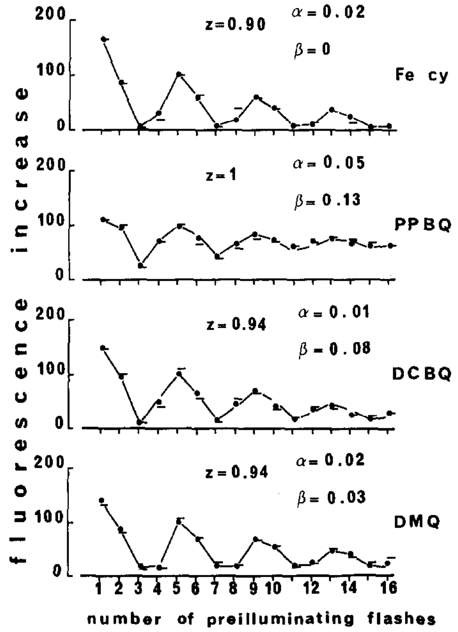

Fig.2. Best least-squares fitting of the oscillation patterns of fluorescence yield shown in fig.1. Fluorescence yields were measured 80 ms after each flash of the series of 16 flashes. A constant level of fluorescence yield was subtracted from the fluorescence yield values. This level was adjusted in order to obtain the best fit. Experimental data are represented by dashes, theoretical data by dots. Results of the fitting are indicated.

(i) The presence of quinones leads to an increase in double hits β on each flash of the sequence. Only in the presence of ferricyanide (fig.2) was the double hit percentage found to be zero. A double hit on the first flash is indistinguishable from some S_2 state centers not yet deactivated in the dark, and thus, cannot be substantiated by our results. By contrast, in the presence of 10 μM PPBQ, a percentage of double hits as high as 17% was determined. The other exogenous quinones induce fewer double hits, from 2 to 8% (table 1 and fig.2). Based on our flash profile [13], part of the electron-transfer rates from Q_A^- to Fe^{3+} must occur in the range 2–3 μs. Thus, we confirm the conclusion of Jursinic and Dennenberg [7] based on oxygen yield measurements that DCBQ facilitates multiple turnovers of reaction centers on each flash.

(ii) Based on the fitting results (fig.2 and table 1), an apparent increase of reactivated centers after each flash is observed in the presence of exogenous quinones. The factor z reflects the proportion of centers that remain active after each flash. This factor is generally equal to z = 0.90, 0.91 in inside-out vesicles in the presence of 0.5 mM ferricyanide, describing a 10–9% loss of centers after each flash of a sequence. This was explained by the progressive reduction of PQ molecules of the pool by flash illumination until the end of the flash series. The amount of reduced PQ molecules and the slow exchange between reaction centers [14] prevent some Q_B in the doubly reduced state Q_B^{2-} from being reoxidized between the flashes of a sequence. As shown in table 1, z increases in the presence of the studied quinones. This result suggests that these electron acceptors may exchange with Q_B^{2-}, responsible for the inactive centers. However, the frequency of substitution between these high-potential quinones and native plastoquinone is not equal. This possibly depends on the shape and orientation of these molecules in the Q_B-binding niche. In the presence of 10 μM PPBQ, z increases considerably, from 0.90–0.91 to 1 (table 1). PPBQ replaces PQ in all of the empty Q_B sites. Centers without bound exogenous quinones, as already explained, have a probability of 10% to become non-functional after each flash of a series. On the other hand, centers with bound exogenous quinones could be 100% active. According to this assumption, the number of centers without bound quinones can be evaluated from the z values. The value z = 1 in the presence of PPBQ indicates that 100% of PS II centers (observed by fluorescence) have bound PPBQ. A smaller increase in z is observed in the presence of DCBQ and DMQ (table 1), corresponding to binding of these quinones in only 40–60% of PS II centers. In comparison with these results, it has been reported that in BBY [15] membranes, 50% of centers show light-induced oxidation of Fe^{2+} at 0°C in the presence of 2 mM PPBQ [9,16,17]. In table 1, the number of double hits after each flash indicates the number of electron transfers from Q_A^- to Fe^{3+} over the period of the flash duration, 3 μs. These transfers after each flash represent about 10%

Table 1

Least-squares fitting results of experimental patterns of fluorescence yield

Electron acceptor	Concentration		z	α	β	S_0	S_1	S_2	S_3
FeCy	0.5 mM	(figs 1a,2)	0.90	0.02	0	30	54	15	1
PPBQ	10 μM		1	0.05	0.17	44.5	34.5	20.5	0.5
	20 μM		0.95	0.01	0.08	40.5	36	23	0.5
DCBQ	10 μM	(figs 1c,2)	0.94	0.01	0.08	37.5	47	14	1.5
	20 μM		0.96	0.03	0.07	37.5	39	23	0.5
DMQ	50 μM	(fig.4)	0.94	0.01	0.02	40	45	2	13
	100 μM	(fig.4)	0.94	0.01	0.05	31	46.5	22	0.5

Flash interval, 730 ms; 0.5 mM ferricyanide added in all samples containing exogenous quinones. In most cases, samples were dark-adapted for 10 min. z, percentage of centers remaining active after each flash of the series; α,β, percentage of misses and double hits, respectively. The S_0–S_3 values denote the S_i concentration in the dark calculated in % assuming that the fluorescence oscillations arise from the S_2 state

(DCBQ, DMQ) and 17% (PPBQ) of the number of centers where an exogenous quinone is bound. The relaxation time for the oxidation of Q_A^- by Fe^{3+} can thus be determined. A $t_{1/2}$ varying from 9 μs (about 3 μs, 50%/17%) for PPBQ to 20 μs for DMQ was found. These values, obtained at pH 6.5 in inside-out thylakoids, are slightly lower than that measured with a 600 ns laser flash at pH 7.5 in BBY membranes, 25 μs [9]. All these results show that PPBQ is the most effective quinone in inducing double hits and in replacing PQ at the Q_B site, while DCBQ and DMQ are less effective. The effectiveness of PPBQ > DCBQ > DMQ approximately follows the extent of Fe^{2+} oxidation observed by EPR spectroscopy [8,9], and rather agrees with the relative position of the midpoint potentials E_m for the Q^-/QH_2 couple, as discussed previously [8,9].

The position of the exogenous quinones in the Q_B-binding niche could affect the rate of electron transfer from Q_A^- to these quinones. According to Petrouleas and Diner [9], the EPR Fe^{3+} spectra in the presence of the different exogenous quinones suggest binding of the quinones to occur close to the Fe^{3+} site, modifying the Fe^{3+} symmetry. The proximity of PPBQ to the Fe^{2+} site may explain the accelerated rate of Q_A reoxidation observed in the presence of PPBQ, as shown in fig.3. The fluorescence decay with a time constant of about 160 μs in the presence of ferricyanide became more rapid after the addition of 10 μM PPBQ (t = 127 μs). This faster rate is consistent with the high efficiency of PPBQ in competing with PQ. The same acceleration in the decay of the fluorescence yield was also observed in the presence of low concentrations of 1,4-benzoquinone [18]. Nevertheless, in the presence of a higher concentration of PPBQ (25 μM) and at lower pH (pH 6.0), Renger et al. [19] observed retardation of the fluorescence decay. This discrepancy could be explained by the fact that, in the presence of 20 μM PPBQ instead of 10 μM, fewer double hits and reactivated centers are formed, as shown in table 1. In our experiments, DCBQ and PPBQ noticeably inhibit the fluorescence oscillations at rather low concentrations (40 μM) with a lag in inhibition probably due to quinone binding in PS II beside the D_1 polypeptide.

Another characteristic effect induced by PPBQ, DCBQ or DMQ is a slower decay of the S_2 state in the dark. In the presence of PPBQ, the EPR S_2 state multiline signal was shown to decay in a slow reaction, which takes several minutes to complete [20,21]. Assuming that the fluorescence oscillations reflect largely the S_2 state [11], the same phenomenon was observed. By varying the interval between the first and second flashes of a series, the decay of S_2 was measured by the change in amplitude of fluorescence oscillations on the fifth flash [11]. In fig.4, the decay of S_2 with time is monophasic with a half decay time of 90 s in the

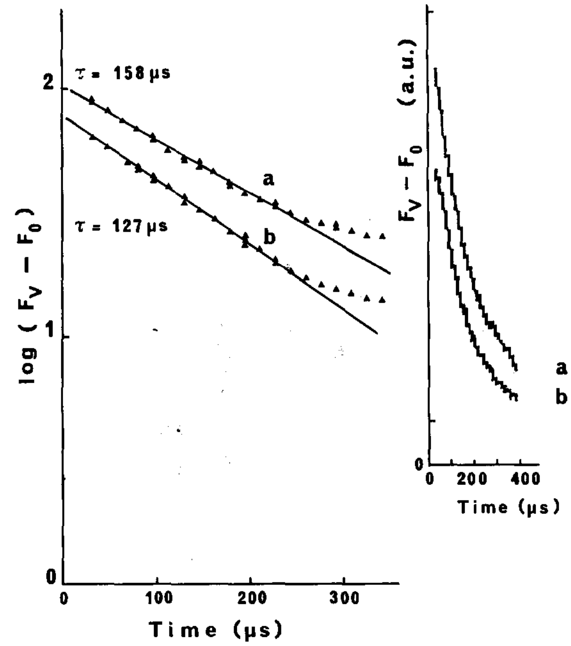

Fig.3. Log plots of the decay of fluorescence, $F_V - F_0$, following the first flash of a series in inside-out thylakoids at pH 6.5, in the presence of (a) 0.5 mM ferricyanide and (b) 0.5 mM ferricyanide and 10 μM PPBQ. The sample was dark-adapted for 10 min. (Inset) Traces of $F_V - F_0$ vs time (μs).

presence of 50 μM DMQ, and 6 min with 100 μM DMQ. Table 1 shows that the concentration of the S_2 state in the dark is increased after the addition of 100 μM DMQ (from 0 to 22%) or the other quinones, but that of the S_3 state remains low or equal to 0. This could indicate that in inside-out thylakoids, the decay of S_3 in the dark does not change fundamentally in the presence of exogenous quinones. In agreement with this assumption, when the time between flashes was varied from 730 ms to 22 s, a marked increase in damping was observed in the presence of 20 μM DCBQ (not shown), probably due to the S_3 decay 22 s after each flash. Furthermore, as shown in figs 1,2 and 4, the fluorescence oscillations are not flattened as would be the case if both S_2 and S_3 states deactivated slowly, which gives rise to almost equal values of the state concentrations in the dark. In PS II-enriched membranes (BBY), both S_2 and S_3 states are stabilized in the dark in the presence of PPBQ [21]. The source of the reducing equivalents needed to deactivate S_2 and S_3 is not known. Recombination seems to take place between the S_2 state and the Q_A^- reduced Signal II_{slow} species, D [22]. In the presence of an exogenous quinone, fewer electrons would be available on the acceptor side for recombination with D^+ and then S_2 [20,21], explaining the stability of S_2.

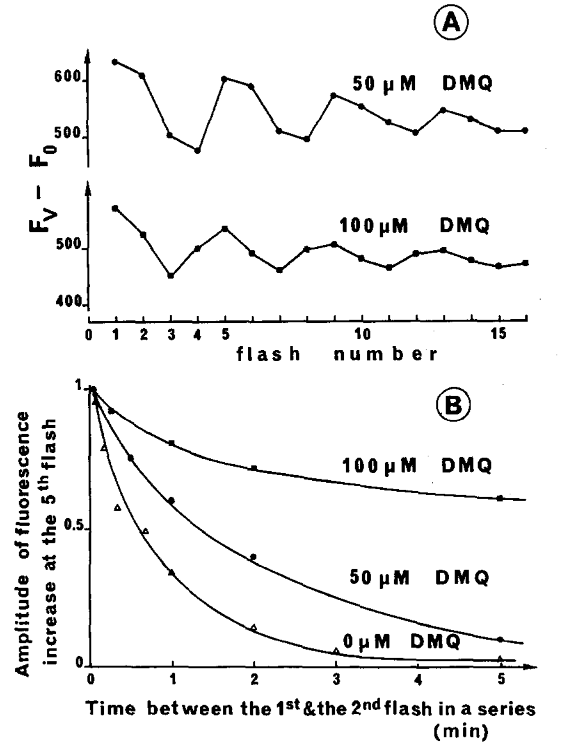

Fig.4. (A) Oscillation patterns of fluorescence yield measured 80 ms after each flash of a series of 16 flashes in the presence of 0.5 mM ferricyanide and 50 μM DMQ or 100 μM DMQ as indicated. (B) Deactivation of the S_2 state observed by fluorescence in the presence of 0.5 mM ferricyanide and 50 μM DMQ or 100 μM DMQ as indicated. The amplitude of fluorescence yield was measured on the fifth flash of a series as a function of the time interval between the first and second flash. Same experimental conditions as in fig.1.

The flash-induced fluorescence oscillations reflect the O_2-evolving system of a homogeneous population of centers [11]. The effects of exogenous quinones on this system are entirely explained by a mechanism which is photoreductant-induced oxidation of Fe^{2+} [8,9].

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