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Photoreductant-induced oxidation of Fe^{2+} in the electron-acceptor complex of Photosystem II

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The ferrous ion associated with the electron acceptors in Photosystem II can be oxidized by the unstable semiquinone form of certain high-potential quinones (phenyl-*p*-benzoquinone, dimethylbenzoquinone and benzoquinone) which are used as electron acceptors. In a flash sequence, alternating oxidation of the iron by the photoreduced semiquinone on odd-numbered flashes is followed by photoreduction of the iron on even-numbered flashes. These reactions are detected by monitoring EPR signals arising from Fe^{3+}. The oxidation of the iron can also occur in the frozen state (−30°C) indicating that the high-potential quinone can occupy the Q_B site. The reaction also takes place when the exogenous quinone is added in the dark to samples in which Q_B is already in the semiquinone form. The inhibitors of electron transfer between Q_A^- and Q_B, DCMU and sodium formate, block the photoreductant-induced iron oxidation. It is suggested that the iron oxidation takes place through the Q_B site. This unexpected photochemistry occurs under experimental conditions routinely used in studies of Photosystem II. Some previously reported phenomena can be reinterpreted on the basis of these new data.

Introduction

Absorption of light energy by P-680, the primary electron donor of Photosystem II, is followed by rapid charge separation in the reaction centre of PS II. P-680 becomes oxidized and a pheophytin molecule bound to the reaction centre protein is reduced. The charge separation is then stabilized by migration of the electron to Q_A and Q_B, two plastoquinone molecules, which act in series. $P\text{-}680^+$ is ultimately reduced by the oxygen-evolving complex, which uses the stored positive charges created by successive turnovers of the PS II reaction centre to oxidize water to oxygen.

A considerable number of observations have been reported in the literature, which have led to the existence of other electron acceptors in PS II being proposed (reviewed in Ref. 1). One of them, Q_{400}, is only functional at high redox potentials ($E_m \simeq 400$ mV) [2,3]; its photoreduction is unaffected by DCMU but its chemical oxidation is inhibited [2,3].

Abbreviations: ANT2p, 2-(3-chloro-4-trifluoromethyl)anilino-3,5-dinitrothiophene; BQ, *p*-benzoquinone; DCBQ, 2,5-dichlorobenzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DMBQ, 2,5-dimethylbenzoquinone; DMSO, dimethylsulphoxide; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Mes, 4-morpholineethanesulphonic acid; Mops, 4-morpholinepropanesulphonic acid; PPBQ, phenyl-*p*-benzoquinone; PQ, plastoquinone; PS II, Photosystem II; Q_A, primary quinone acceptor; Q_B, secondary quinone acceptor; Q_{400}, the high potential acceptor; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

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From different lines of evidence, Q_{400} was reported recently by Petrouleas and Diner [4] to be Fe^{3+}, the oxidized form of Fe^{2+} which is associated with the quinone acceptors Q_A and Q_B. When PS II membranes were incubated with ferricyanide, a treatment which raises the redox potential sufficiently for Q_{400} to become functional, EPR signals at $g = 8$ and $g = 6$ were observed, which were attributed to Fe^{3+}. These signals have also been seen by Nugent and Evans [5], who reported that these signals disappeared upon illumination at low temperature [5].

In this work, EPR observations are reported which confirm the assignment of Q_{400} to Fe^{3+}, the iron associated with the acceptor side of PS II. Furthermore, it is shown that in the presence of high potential exogenous quinones (such as phenyl-*p*-benzoquinone (PPBQ), dimethylbenzoquinone (DMBQ) and benzoquinone (BQ)), the formation of $Fe^{3+}(Q_{400})$ can occur due to oxidation by the unstable semiquinones generated by light-driven electron transfer. Under flash illumination, oscillations of iron (Q_{400}) oxidation and reduction occur. The mechanism of this unexpected photoreaction is investigated, and its significance to other data in the literature is discussed.

Materials and Methods

O_2-evolving PS II membranes were prepared from market spinach as already described [6,7] and resuspended at high concentration (approx. 12 mg Chl/ml) in a buffer containing 400 mM sucrose/20 mM Mes (pH 6.0)/15 mM NaCl/5 mM $MgCl_2$.

For pH experiments, PS II membranes were resuspended at 2 mg/ml in 15 mM NaCl/10 mM $MgCl_2$/50 mM buffer. Buffers used were: Mes (pH 5.5, pH 6.0, pH 6.5), Mops (pH 7.0), Hepes (pH 7.5) and Tricine (pH 8.0). After centrifugation for 30 min at 35 000 × *g*, the pellet was resuspended in 400 mM sucrose/15 mM NaCl/5 mM $MgCl_2$/20 mM buffer.

EPR samples in calibrated quartz tubes were incubated in darkness for 20 min at 20°C before being frozen to 200 K, then to 77 K. Additions to the EPR samples (see legends to figures) were made in near darkness but before the dark-incubation, unless otherwise stated.

For flash experiments, the samples were diluted to 3 mg/ml so that the flash was saturating [8].

EPR spectra at X-band were recorded at low temperature using a Bruker ER-200-tt spectrometer fitted with an Oxford Instruments helium cryostat and temperature-control system. EPR spectra with 32 G modulation amplitude were recorded with a Bruker ER-41-VR water-cooled cavity. For other modulation amplitudes (see legends to figures), the standard TE_{102} mode cavity was used. In some cases, a Tracor-Northern 1710 computer was coupled to the EPR spectrometer for averaging and substracting the spectra.

Illumination at 200 K was provided in an unsilvered Dewar flask containing an ethanol-solid CO_2 mixture, using a 800 W projector. The same set-up was used for illumination at 77 K, but the flask contained liquid N_2. In both cases infrared radiation was cut off by 2 cm of water and three Calflex filters.

Excitation with one or more laser flashes was provided by a Quantel-Nd-YAG Laser giving a 100 mJ, 15 ns pulse at 530 nm. After the flash was given, the sample was rapidly frozen to 200 K and then stored at 77 K.

In some experiments, PPBQ was added just after the flash. The sample was frozen after a mixing and incubation period of 30 s at 4°C in complete darkness.

In other experiments (see legends to figures) EPR samples were illuminated at 77 K and the EPR spectra were recorded. The samples were then thawed to 18°C. PPBQ was immediately added to the sample. After a mixing and incubation period of 30 s, the sample was frozen and EPR spectra recorded.

DCMU, PPBQ, BQ, DCBQ and DMBQ (Sigma Chemicals) were dissolved in DMSO as 20 mM stocks.

Results and Discussion

When dark-adapted PS II membranes are illuminated by a series of flashes at room temperature in the presence of PPBQ (1 mM), EPR signals are photoinduced, the amplitudes of which depend on the number of flashes given. A multiline signal and a $g = 4.1$ signal attributed to the manganese

cluster of the oxygen-evolving complex in the S_2 oxidation state have maximal amplitudes after 1 and 5 flashes [9]. In the same samples in which oscillations of the S_2 signals were observed, other EPR signals were present, the amplitude of which also depended on flash number. Fig. 1A (solid lines) shows that EPR signals at $g \simeq 7.9$ and $g \simeq 5.3$ are photoinduced on the first, third and fifth flashes and that these signals are not present in the dark nor after even numbered flashes. Fig. 1B is a plot of the amplitude of the $g \simeq 7.9$ signal photoinduced by a series of flashes given at room temperature. It is clear that the amplitude of this signal oscillates with a periodicity of two with maxima on odd-numbered flashes. Fig. 1A (broken lines) also shows that further illumination given at 200 K results in the disappearance of these signals whenever they are present. When no PPBQ is added to the PS II membranes, no $g \simeq 7.9$ and $g \simeq 5.3$ signals are photoinduced, although damped oscillations in the amplitude of the S_2 multiline signal are still observed (not shown).

Petrouleas and Diner [4] recently reported the observation of EPR signals at around $g \simeq 8$ and $g \simeq 6$ in PS II membranes incubated with 2 mM ferricyanide. These signals disappeared upon reduction by ascorbate or illumination at 200 K [4], thus reflecting the oxidation of a PS II component. This component was shown by Mössbauer spectroscopy to be Fe^{3+} [4]. To test whether their observations and those reported here reflect the oxidation of the same component, PS II membranes were incubated with 2 mM ferricyanide. Fig. 1C shows that, in the dark, EPR signals at $g = 7.9$ and $g = 5.3$ are observed with amplitudes similar to those found under the conditions of Fig. 1A. Excitation with a flash completely removes the ferricyanide-induced signals (Fig. 1C solid line). These results indicate that Fe^{3+} is formed by chemical oxidation in the dark and that it is fully reduced by a single flash at room temperature. Thus the data in Fig. 1A indicate that excitation of PS II membranes with a series of flashes in the presence of PPBQ results in alternating photooxidation and photoreduction of iron.

When formed by one flash in the presence of PPBQ, Fe^{3+} remains oxidized for a few minutes, as measured by the amplitude of the EPR signal at $g \simeq 7.9$; after 20 min incubation in the dark, the photoinduced $g = 7.9$ and $g = 5.3$ signals were nearly absent.

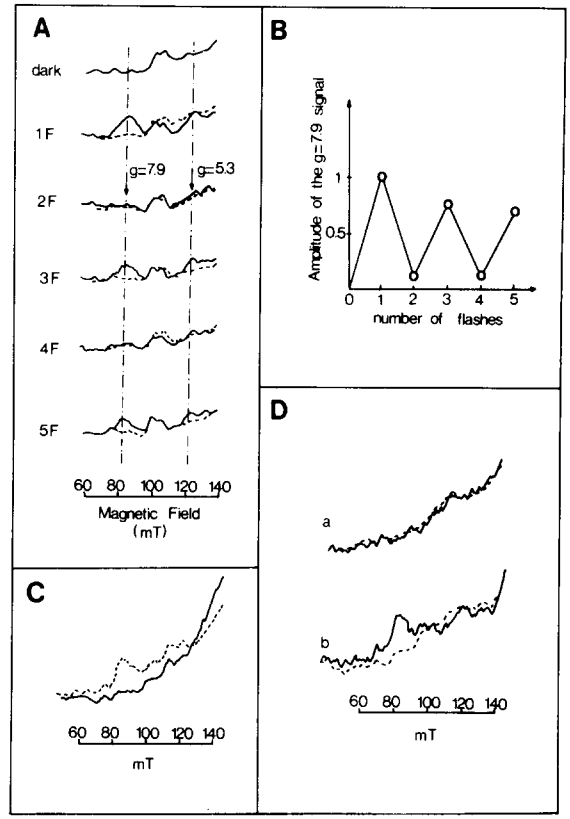

Fig. 1. (A) EPR spectra of PS II membranes incubated with 1 mM PPBQ, generated by a series of flashes given at room temperature. Solid lines: after n flashes. Dashed lines: after further illumination for 2 min at 200 K. (B) Amplitude of the $g = 7.9$ signal detected after a series of flashes at room temperature. (C) EPR spectra of PS II membranes incubated with 2 mM ferricyanide in the dark (dashed line) and after excitation with a laser flash at room temperature (solid line). (D) Effect of 1 mM PPBQ on the EPR signals at $g = 7.9$ and $g = 5.3$ in PS II membranes incubated with 100 μM ANT2p. (a) 100 μM ANT2p; (b) ANT2p + PPBQ. Dashed lines: dark-adapted PS II membranes. Solid lines: after excitation with one laser flash followed by incubation at 20 °C for 10 s. Instrument settings: frequency, 9.4 GHz; modulation amplitude, 32 G; temperature, 4.8 K; microwave power, 32 mW.

Petrouleas and Diner attributed the chemically oxidized iron to the iron associated with the quinone electron acceptors [4]. However, since the photooxidation of an acceptor side component seems somewhat paradoxical, a donor side role needs to be considered. The following observa-

tions are relevant to this question. (i) When no PPBQ is added to the PS II membranes, no photooxidation of Fe^{2+} occurs, although damped S_2 EPR signal oscillations are still observed with a series of flashes (not shown). (ii) Incubation of PS II membranes with ferricyanide results in the oxidation of Fe^{2+} to Fe^{3+}, as demonstrated by the appearance of the EPR signals at $g = 7.9$ and $g = 5.3$. Further excitation with one flash results in its complete photoreduction, although S_2 EPR signals with amplitudes similar to those in a control sample are still observed. (iii) Incubation of PS II membranes with ANT2p, which catalyses the deactivation of the donor side of PS II [10], has no effect on the flash-induced Fe^{3+} EPR signal (Fig. 1D), although complete deactivation of S_2 occurred, as monitored by the disappearance of the S_2 multiline signal (not shown). These points clearly indicate that the Fe^{3+} signal is not associated with the redox state of the donor side of PS II. Thus, it is concluded that the observations reported above reflect the oxidation of Fe^{2+} associated with the acceptor complex of PS II.

The requirement of PPBQ for the photooxidation of Fe^{2+} at room temperature suggests a possible mechanism. After a flash, the photoinduced Q_A^- is reoxidized by PPBQ (possibly via Q_B). The semiquinone form of PPBQ thus produced is unstable and extracts an electron from Fe^{2+} to form Fe^{3+} and $PPBQH_2$. This mechanism is a reductant-induced oxidation and similar phenomena are known in other electron transfer systems in which unstable semiquinone formation occurs [11]. According to this mechanism, the disappearance of the Fe^{3+} signals on even-numbered flashes is simply due to photoreduction of Fe^{3+} probably by Q_A^- to form Fe^{2+}. To test and refine this explanation a number of experiments have been performed.

Illumination of PS II membranes at 77 K results in the photogeneration of Q_A^-Fe, largely at the expense of cytochrome *b*-559, and further electron transfer beyond Q_A is blocked at this temperature [12]. Thus illumination at 77 K followed by warming to room temperature is equivalent to a single turnover on the acceptor side of PS II without significant change in the physiological charge accumulation system of the donor side [13]. Such an experiment also allows much higher concentrations to be used than could be saturated by flash illumination.

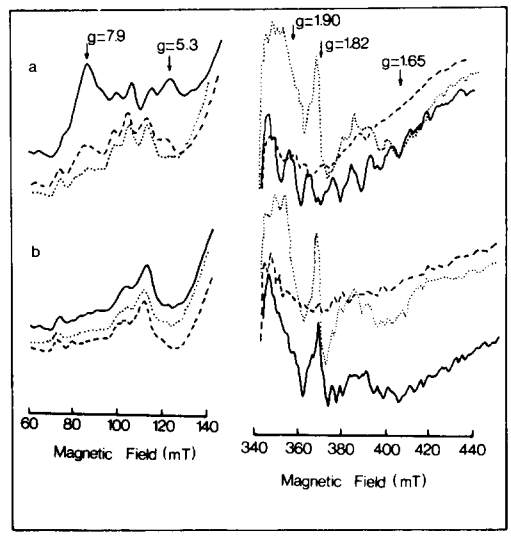

Fig. 2. EPR spectra of PS II membranes incubated for 10 min with 1 mM PPBQ (a) or with no addition (b). The spectra shown are of dark-adapted (dashed lines) PS II membranes, after 20 min illumination at 77 K (dotted lines) and after a further incubation of 15 s at 18°C (solid lines). Instrument settings: left spectra (low-field range): modulation amplitude, 32 G; temperature, 4.5 K; microwave power, 5 mW; right spectra (high-field range): modulation amplitude, 20 G; temperature, 4.5 K; microwave power, 32 mW.

Fig. 2 (dashed lines) shows that in a dark-adapted sample incubated with PPBQ, a small signal at $g = 7.9$ is present, while no signal is visible in the control sample. Illumination for 20 min at 77 K results in the loss of the $g = 7.9$ signal in the sample treated with PPBQ, indicating that the oxidized iron is photoreduced; no change is visible in the control sample (dotted lines). Photogeneration of Q_A^-Fe is demonstrated by the appearance of well-resolved signals at $g = 1.82$ [14,15] and $g = 1.90$ [16], with amplitudes similar in both samples (the extent of Q_A^-Fe formed in the PPBQ-containing sample is 100%, since more than one electron equivalent is available from the donor side at this temperature (e.g., Ref. 17)). Incubation of these samples for 15 s at 18°C results in the appearance of large signals at $g = 7.9$ and $g = 5.3$ in the PPBQ-containing sample, while no change can be observed in the control sample (solid lines). In the PPBQ-treated sample, the

Q_A^-Fe signals are completely lost upon incubation at 18°C (solid line). In the control sample, a significant proportion of the $g = 1.82$ signal remains after 15 s incubation at 18°C, the $g = 1.90$ resonance is sharpened and its maximum is shifted towards $g = 2.0$ (Fig. 2b, solid line). Such a change in the semiquinone iron signals has already been observed under similar circumstances and has been attributed to the formation of Q_B^-Fe by electron transfer from Q_A^- [18]. These results demonstrate that electron transfer beyond Q_A^- is required for photooxidation of Fe^{2+} to occur. In the control sample, Q_B^-Fe (and perhaps some Q_A^-Fe) is present in the dark after thawing. When PPBQ is present, it accepts the electron and the iron is oxidized by the unstable semiquinone form of PPBQ.

It is also of note in the experiments shown in Fig. 2 that small amounts of the $g = 4.1$ (not shown) and multiline signals are photoinduced at 77 K. Both of these signals arise from the S_2 state and warming in the dark converts the former to the latter [17,19]. The multiline signal in Fig. 2 generated by thawing the samples preilluminated at 77 K is an example of this effect. When no PPBQ is present, S_2 decays by recombination with Q_B^- [13] with a half-time of 22 s [20]. When PPBQ is used as an acceptor, no Q_B^- is present, and consequently S_2 is expected to decay more slowly. Thus a larger S_2 multiline signal is present in the sample containing PPBQ after thawing than in the control. This is only a small proportion of the S_2 multiline signal which can be generated by illumination at 200 K. A previous estimation of S_2 formation by 77 K illumination was 5–10% [13].

At −30°C some electron transfer from Q_A^- to Q_B takes place [12]. The shape change in the semiquinone iron EPR signal attributed to Q_B^-Fe formation occurs when samples illuminated at 77 K are warmed to this temperature [18]. In the presence of PPBQ, warming of a sample preilluminated at 77 K to −30°C resulted in the formation of the Fe^{3+} signal (not shown). This indicates that oxidation of Fe^{2+} by $PPBQ^-$ can take place in the frozen state and suggests that PPBQ can occupy the Q_B site and that the oxidation of Fe^{2+} takes place from the Q_B site itself.

It is noted above that some Fe^{3+} is present in the dark in the sample incubated with PPBQ (Fig. 2a). As some Q_B^- is present in dark-adapted PS II membranes, the addition of PPBQ oxidizes Q_B^-. $PPBQ^-$ then oxidizes Fe^{2+}. To verify that PPBQ reduction (and subsequent Fe^{2+} oxidation) can take place using the electron from Q_B^-, experiments were performed in which PPBQ was added after the photoreduction of Q_B to Q_B^- had already occurred. Fig. 3 shows two different methods by which this was achieved. In Fig. 3a excitation with one flash at 0°C of PS II membranes does not produce the $g = 7.9$ signal. However, the addition of PPBQ in the dark after the flash results in the oxidation of Fe^{2+} to Fe^{3+}, as demonstrated by the generation of the EPR signal at $g = 7.9$. In the absence of PPBQ, illumination at 77 K followed by incubation at room temperature forms Q_B^-Fe (see above). Addition of PPBQ at 0°C in the dark after this treatment generates Fe^{3+} (Fig. 3b), while the semiquinone-iron signals are lost (not shown, but see Fig. 2). Clearly PPBQ can be reduced by

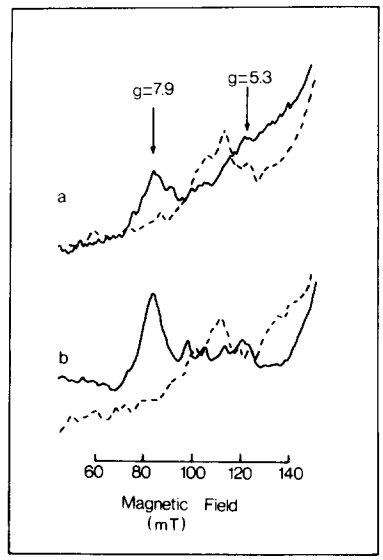

Fig. 3. EPR signals induced by addition of PPBQ to preilluminated PS II membranes. (a) Dashed line: PS II membranes (3 mg Chl/ml) adapted in the dark; solid lines: after excitation with a laser flash PPBQ was added immediately to a final concentration of 1 mM and mixed in the EPR tube. This was done at 0°C and the time taken was about 40 s. The EPR tube was then frozen and EPR spectra taken. (b) Dashed line: PS II membranes (10 mg Chl/ml) illuminated for 20 min at 77 K and then incubated in the dark for 30 s at 18°C. Solid line: after a further 30 s dark incubation at 18°C during which PPBQ was added and mixed with the sample to a final concentration of 1 mM. Instrument settings were as in Fig. 1.

Q_B^-; the $PPBQ^-$ formed then oxidizes Fe^{2+}. This may occur after $PPBQ^-$ replaces Q_B in the Q_B site.

DCMU blocks the reoxidation of Q_A^- by Q_B, by competing for the Q_B binding site on the PS II reaction centre protein complex [21,22]. It was observed that the PPBQ-induced photooxidation of iron was inhibited by DCMU (not shown). This effect is probably due to inhibition of electron transfer to Q_B (or to PPBQ in the Q_B site). Extending this idea, it might be suggested that the previous observation that DCMU inhibits Q_{400} oxidation may be partially due to the fact that ferricyanide oxidation of Q_{400} also takes place through the Q_B site.

Fig. 4 shows that formate also prevents iron oxidation in the presence of PPBQ. Transfer of electrons from Q_A^- to Q_B is slower in formate-treated thylakoids [1]; however, in PS II membranes the nature of the formate lesion is not well characterized. In the experiment shown in Fig. 4 under the conditions where Q_B^- would be expected to be formed, no specific spectral change in the semiquinone-iron signal shape which could be associated with Q_A^- to Q_B electron transfer could be detected. A possible interpretation is that the formate lesion in this preparation is like that caused by DCMU. The lack of PPBQ induced Fe^{2+} oxidation in formate-treated samples could be simply due to this electron-transfer blockage.

The semiquinone iron signal is relatively long lived at room temperature, when formed by low temperature illumination in the presence of sodium formate. However, its decay is accelerated in the presence of PPBQ (see Fig. 4). If the formate is acting as a DCMU-type block this result would indicate that Q_A^- is oxidized by PPBQ at a site different from Q_B.

An alternative explanation for these results is that Q_B^-Fe is being formed in the presence of formate (which under these conditions gives rise to an EPR signal undistinguishable to that of Q_A^-Fe). PPBQ is then reduced by Q_B^-, but does not oxidize Fe^{2+} due to a formate induced modification of the Q_B site or a change in the E_m of Fe^{2+}/Fe^{3+}. The observation that formate inhibits iron oxidation is relevant to the report [23] that no Q_{400}-induced double hit was observed when formate was present before ferricyanide treatment.

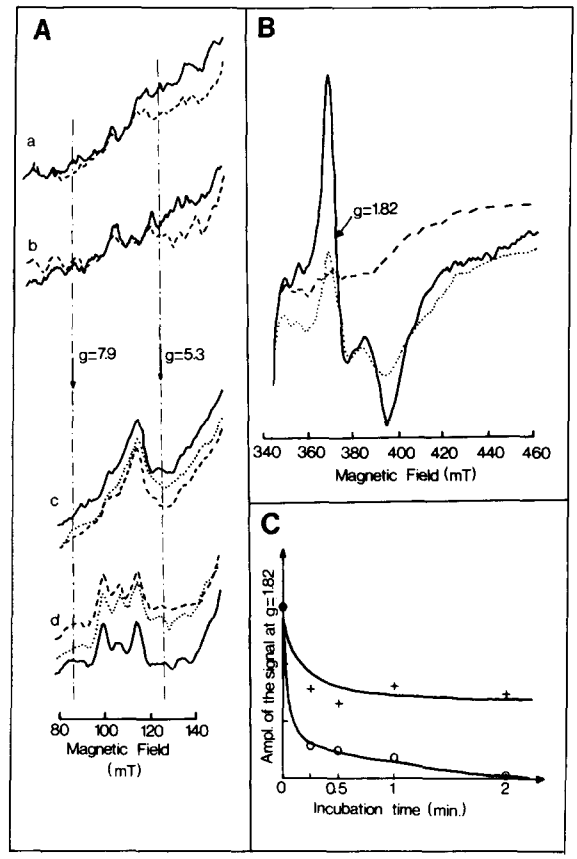

Fig. 4. (A) Effect of sodium formate on the reductant-induced oxidation of Fe^{2+} in PS II membranes. (a) and (b), dashed lines: dark-adapted PS II membranes (3 mg Chl/ml); solid lines: after excitation with one flash at 0°C. (c) and (d), dashed lines: dark-adapted PS II membranes (10 mg Chl/ml); solid lines: after 20 min. illumination at 77 K; dotted lines: after a 30 s dark incubation at 18°C. All samples contained 100 mM sodium formate. (a) and (c); no further addition; (b) and (d): +1 mM PPBQ. Instrument settings were as in Fig. 1. (B) EPR signals of Q_A^-Fe induced under the conditions of Fig. 4Ac (100 mM formate, no PPBQ). Instrument settings: modulation amplitude, 32 G; temperature, 4.6 K, microwave power, 32 mW. (C) Amplitude of the EPR signal at $g = 1.82$ in PS II membranes illuminated for 20 min at 77 K, and further incubated for various times in darkness at 18°C. Crosses: 100 mM formate; circles: 100 mM formate+2 mM PPBQ. Instrument settings are as in B.

This might be reinterpreted as being due to inhibition of Q_{400} oxidation rather than to formate inhibiting its photoreduction, as originally proposed [23].

A number of quinones are commonly used as electron acceptors in PS II. In Fig. 5 we have

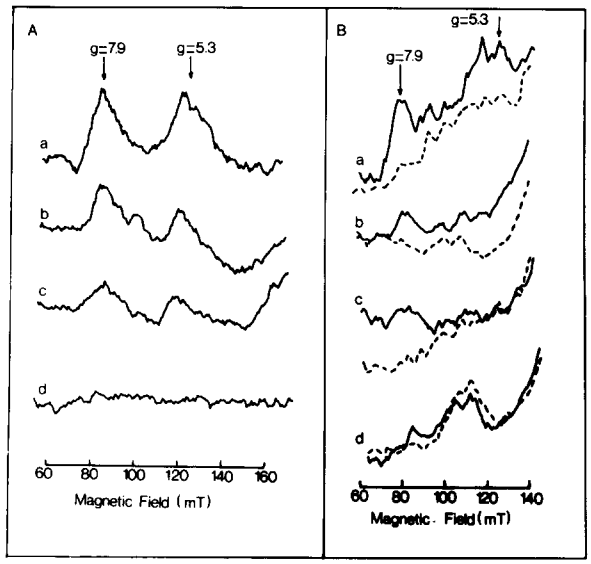

Fig. 5. EPR signals of Fe^{3+}, produced in PS II membranes incubated with various exogenous quinones. (A) Spectra of 20 min illuminated membranes at 77 K are substracted from those obtained after a further dark-incubation at 18°C for 30 s (average of two spectra). (B) Dashed lines: dark-adapted PS II membranes; solid lines: after excitation with one flash at 0°C. (a) PPBQ, 1 mM; (b) BQ, 1 mM; (c) DMBQ, 1 mM; (d) DCBQ, 1 mM; Instrument settings were as in Fig. 1.

tested those most often used, to determine if Fe^{2+} oxidation occurs. Data from single flash experiments and from more concentrated samples given a single turnover by low-temperature illumination are shown. It can be seen that PPBQ is the most effective quinone used, while BQ and DMBQ are slightly less effective. Dichlorobenzoquinone (DCBQ) does not seem to work. In addition, the endogenous electron acceptor, plastoquinone (PQ) cannot induce iron oxidation. Table I shows that the midpoint-potentials in solution of the most likely couple involved (i.e., Q^-/QH_2) are not sufficient to explain the reactivities of the quinones. Other factors like hydrophobicity, stabilization of the semiquinone in the Q_B site and/or protonation may be involved to explain the data. More work needs to be done to understand the differential effects of the quinones.

TABLE I

MIDPOINT POTENTIALS (VS. NHE) FOR THE REDOX COUPLE (Q^-/QH_2) OF VARIOUS QUINONES

Data are calculated from Ref. 24. That for PPBQ is assumed to be the same as for 2-methyl-*p*-benzoquinone (see Ref. 29).

	BQ	DMBQ	DCBQ	PPBQ	PQ
$E_m(Q^-/QH_2)$ at pH 6.0 (mV)	611	548	515	573	517

Other data indicate that the reactions are complicated by binding site effects. If the Q^-/QH_2 couple were simply operating, one would expect a 120 mV per unit decrease in E_m as the pH was raised. Since the $Q_{400}{}^{ox}/Q_{400}{}^{red}$ couple shows a 60 mV per pH unit dependence [3], it might have been expected that raising the pH should lead to a loss of iron oxidation. Preliminary experiments have shown that in the presence of PPBQ the iron oxidation takes place at pH 8.5. Paradoxically, at pH 5.5 the extent of Fe^{2+} oxidation was drastically decreased (only 20% was observed). This might be interpreted as an effect of protein protonation inhibiting the iron oxidation, perhaps due to restricted access to the iron or to redox effects on the iron. Wraight [25] suggested the existence of a pK at pH 5.2 which resulted in no proton release concomitant with Q_{400} (iron) oxidation. Such a pK could be related to loss of iron oxidation at low pH. Future studies of the structural and redox properties required for iron oxidation may provide information on the properties of the Q_B binding site.

The chemical oxidation of Q_{400} (Fe^{3+} formation) in PS II has been associated with low DCMU binding at high pH [25] and phase shifts in the $Q_B{}^-$ oscillation [26]. Here we show that the use of certain quinones as electron acceptors (PPBQ, BQ and DMBQ, but not DCBQ) gives rise to oscillations in formation of Fe^{3+} (oxidized Q_{400}). Thus oscillations of the appearance of a DCMU-insensitive acceptor would be predicted. Indeed some phenomena already published in the literature indicate that such effects have been observed. Lavergne [27] showed that chloroplasts which had received a flash in the presence of BQ contained two electron acceptor equivalents when DCMU was added after the flash. The extra acceptor was interpreted as being due to binding of BQ^- which could undergo DCMU insensitive reduction. From the present work it seems that this effect should be reinterpreted as a BQ^- induced oxidation of iron.

This is supported by the fact that kinetics of electron transfer from Q_A^- to the extra acceptor reported by Lavergne was 100 μs (Ref. 27, see also Ref. 28), a value predicted for Q_A^- to Q_{400} electron transfer measured earlier [26].

Assuming this reinterpretation to be correct, Lavergne's work [27] also provides information on the reductant-induced oxidation reaction. Firstly, it shows that the effect occurs in chloroplasts. We have been unable to detect EPR signals attributable to Fe^{3+} in chloroplasts due to the large signals present in the dark in the low-field part of the spectrum. Secondly, some idea of the rate of the iron oxidation can be obtained from Lavergne's experiments showing that the rate of iron reduction is determined by Q_B^- oxidation by the benzoquinone and that this is dependent upon the concentration of the benzoquinone. Here, however, the iron oxidation is observed at $-30°C$ and although a slow diffusion of the quinones might occur at this temperature, a more likely explanation is that PPBQ binds in the Q_B site in competition with PQ. If this is the case, it would be predicted, in contrast with Lavergne's data, that the concentration of the quinone would not be rate limiting. This discrepancy could be due to the nature of the quinone, the concentration of the quinone or the type of membrane preparation used.

This report of unexpected photochemistry in the PS II reaction centre is important to workers using these quinones as exogenous electron acceptors in spectroscopic studies. In addition it provides a new reaction by which quinone binding in the PS II reaction centre may be studied.

While this work was in progress, Petrouleas and Diner (personal communication) observed iron oxidation in BQ-treated samples which had been thawed after low temperature illumination. Their interpretation of this effect is in line with that reported here.

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