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The influence of the quinone-iron electron acceptor complex on the reaction centre photochemistry of Photosystem II

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A correlation is demonstrated between the loss of the $Q_A^-Fe^{2+}$ EPR signal and the ability to photoinduce the radical-pair-recombination triplet state in Photosystem II. The $Q_A^-Fe^{2+}$ signal is diminished by procedures which are thought to reduce the semiquinone by a further electron: (1) low quantum yield photoreduction in the presence of sodium dithionite at room temperature; (2) chemical reduction in the dark by sodium dithionite at pH 7.0. The chemical reduction process is extremely slow ($t_{1/2} \approx 5$ h) but can be accelerated ($t_{1/2} \approx 1.5$ h) by the presence of the redox mediator, benzyl viologen. In redox titrations at pH 7.0 the $Q_A^-Fe^{2+}$ signal disappears with an irreversible transition at potentials lower than −350 mV. The ability to observe the triplet signal shows a corresponding potential dependence. The variations in the amplitude of the triplet EPR signal match variations in triplet yield measured by flash absorption spectroscopy at low temperature. From these observations the following conclusions are drawn: (1) The redox titration data that led to the suggestion that an extra component functions between pheophytin and $Q_A^-Fe^{2+}$ (Evans, M.C.W., Atkinson, Y.E. and Ford, R.C. (1985) Biochim. Biophys. Acta 806, 247–254) can probably be explained instead by the second reduction of $Q_A^-Fe^{2+}$. (2) The variable yield of triplet and of $P680^+Ph^-$, and possibly the lifetime of the latter, which have been reported in the literature probably reflect, at least in part, different amounts of native $Q_A^-Fe^{2+}$ remaining in the various preparations used. From considerations of the literature, an increase in quantum yield of charge separation is thought to occur upon the second reduction of $Q_A^-Fe^{2+}$. The most likely explanation for this is the disappearance of an electrostatic interaction between $Q_A^-Fe^{2+}$ and $P680^+Ph^-$ as $Q_A^-Fe^{2+}$ becomes further reduced. Other factors which may influence or be responsible for these phenomena and comparisons with the primary photochemistry in purple bacteria are discussed. In addition the relevance of these observations to the lesions involved in photoinhibition is pointed out.

Introduction

In the reaction centre of purple photosynthetic bacteria the first detectable radical pair formed after excitation by light is P^+BPh^-. P, the primary electron donor, is a special pair of bacteriochlorophyll molecules and BPh, the primary electron acceptor, is a bacteriopheophytin molecule (reviewed in Refs. 1, 2). If further forward electron transfer is blocked by removal or reduction of the subsequent electron acceptor, a quinone molecule (Q_A), spin dephasing followed by charge recombination takes place resulting in the formation of a triplet state of P. The yield of formation of the triplet state of P is close to 100% at liquid helium temperature [3]. At ambient temperature other recombination pathways occur and the triplet yield is only approx. 15% [1].

In Photosystem II (PS II), similar photochemistry is thought to take place [4] (reviewed in Ref. 5). However, a number of observations have led to suggestions that PS II differs significantly from purple bacteria with regard to the number and nature of the electron acceptors (e.g., Ref. 6). In a detailed study of the triplet EPR signal it was shown that the triplet signal was very small until the potential was decreased to below −350 mV [6]. In the same work it was shown that Q_A^-, measured as the $Q_A^-Fe^{2-}$ signal at $g \approx 1.85$ [7], was fully formed

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Abbreviations: BPh, bacteriopheophytin; Mes, 4-morpholineethanesulphonic acid; P, the primary electron donor in purple bacterial reaction centres; P680, the primary electron donor in Photosystem II; Ph, pheophytin; PS II Photosystem II; Q_A, the first quinone electron acceptor; Q_B, the second quinone electron acceptor.

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at much higher potentials [6]. The non-correlation of triplet formation with the Q_A/Q_A^- transition led to the suggestion that an extra component, U, was present which functioned as an electron acceptor in PS II between Ph and Q_A [6,8].

There is plenty of precedence for 'extra acceptors' in this photosystem (reviewed in Ref. 9), yet the weight of evidence, both spectroscopic [5,9] and biochemical [10,11], pointing to a close analogy with the bacterial reaction centre led us to look for other explanations for this effect.

The quinone in the Q_A site in the reaction centres of PS II and purple bacteria shows redox chemistry which is very different from that of quinones in solution. The quinone is tightly bound to the protein and under normal circumstances undergoes a single electron reduction forming the semiquinone. In purple bacteria it was shown that, in the presence of reducing conditions and an efficient electron donor, illumination at room temperature resulted in the low quantum yield reduction of Q_A to form the fully reduced quinol [12,13]. This second reduction of Q_A can be achieved by chemical reductants in the dark in a slow, quasi-irreversible reaction [14]. Normal Q_A function could be reestablished only after the system was fully reoxidized [14]. This behaviour may be rationalized as follows: upon double reduction, QH_2 is lost from the site; upon raising the potential, QH_2 in solution undergoes a two-electron oxidation forming the fully oxidized quinone which can then rebind in the Q_A site.

In PS II, a similar light-driven double reduction of Q_A has been reported [15,16]. In this work we tested the idea that double reduction of Q_A could influence the yield of PS II reaction centre triplet formation.

A preliminary report of these results has appeared elsewhere [17].

Materials and Methods

PS II-enriched thylakoid membrane fragments were prepared from spinach as described earlier [18] using the modifications in Ref. 19. In some experiments, the membranes were washed with Tris before use. Membranes (0.5 mg chlorophyll/ml) were exposed to room light for 30 min at 0 °C in the presence of 0.8 M Tris (pH 8.2)/1 mM EDTA/1 mM EGTA. The Tris-treated membranes were pelleted and washed once in a buffer containing 50 mM Mops (pH 7.0)/10 mM NaCl/5 mM $MgCl_2$ and resuspended in the buffer used for redox titrations (50 mM Mops (pH 7.0)/10 mM NaCl/5 mM $MgCl_2$/30% ethylene glycol/1 mM EDTA). EPR samples (about 250 μl) were in calibrated quartz tubes (3 mm internal diameter). For time-course experiments, incubations were done in the EPR tube at room temperature in darkness. At a given time, the incubation was stopped by freezing the samples, the spectra were recorded, the sample thawed and the incubation allowed to continue at room temperature. It was demonstrated that multiple freeze–thaw cycles under these conditions had no noticeable effect on the EPR signals monitored.

Redox titrations of Tris-washed samples (about 5 mg chlorophyll/ml) were performed in near darkness at 20 °C essentially as described by Dutton [20]. The following redox mediators were used: phenyl-*p*-benzoquinone, indigo tetrasulphonate, indigo disulphonate, 2,6-dichlorophenol indophenol, Methylene blue, anthroquinone 2-sulphonate, anthroquinone 2,6-disulphonate, Saphranine T and Neutral red, all at 50 μM; in addition, benzyl viologen and methyl viologen were present at 20 μM.

Samples for flash absorption kinetic studies were transfered from EPR tube to the optical cuvette under argon. Except for the chlorophyll concentration (2.9 mg chlorophyll/ml for EPR and 50 or 100 μg chlorophyll/ml for absorption measurements) and the benzyl viologen concentration (34 μM for EPR and 0.7 μM or 1.4 μM for absorption) the other conditions (buffer, cryoprotectant, temperature) were identical for both kinds of measurement (see legends).

White light from an 800 W tungsten projector was used for illuminating samples after passing through 2 cm of water and two calflex heat filters (Balzers). For room temperature illuminations ($\approx 7000\ \mu E \cdot m^{-2} \cdot s^{-1}$) samples were maintained at 20 °C in a water-bath. When illuminated in the EPR cavity the intensity was $\approx 16000\ \mu E \cdot m^{-2} \cdot s^{-1}$ at the cavity window. EPR spectra were recorded using a Bruker 200 X-band spectrometer fitted with an Oxford Instruments cryostat and temperature control system.

Absorption measurements were made using a flash kinetic spectrophotometer described earlier [21], except that the detector was a PIN 10 photo-diode equipped with a 1 MHz bandwidth amplifier. Some measurements were also made using a different spectrophotometer as described previously [22]. Samples were cooled to close to liquid N_2 or liquid He temperature using S.M.C. cryostats.

Results

To obtain reproducible conditions for low-temperature illumination experiments, relatively dilute samples (3 mg Chl/ml) were used frozen in 30% ethylene glycol. To obtain measureable EPR signals from $Q_A^-Fe^{2+}$ under these conditions sodium formate was added, since this results in a greater than 10-fold increase in the EPR signal at $g = 1.85$ [23] probably due to binding of this chemical directly to the iron [5]. When such samples were reduced by sodium dithionite they exhibited easily detectable EPR signals at $g = 1.85$ typical of the formate-modified $Q_A^-Fe^{2+}$ (Fig. 1c). No detectable triplet EPR signal could be observed in these samples when

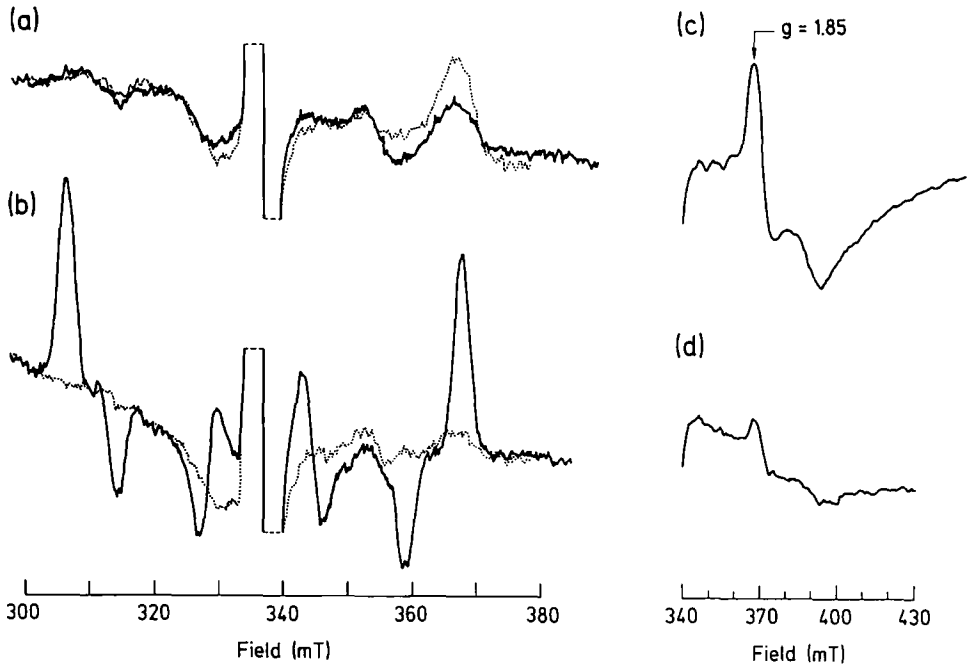

Fig. 1. The influence of room temperature illumination on dithionite-reduced PS-II-enriched thylakoid membranes monitored by EPR. The samples (3 mg chlorophyll/ml) were in 25 mM Mes (pH 6.5)/0.3 M sucrose/10 mM NaCl/5 mM $CaCl_2$/30% ethylene glycol/1 mM EDTA/40 mM dithionite. Spectra a and c were recorded in a sample incubated in the dark for 20 min after dithionite addition. Spectra b and d were recorded in the same sample but after thawing and a total of 12 min (i.e., six periods of 2 min to avoid sample heating) illumination at 20 °C followed by dark adaptation for 25 min. Triplet spectra (a and b) were recorded under illumination (solid lines), dotted lines show dark spectra. EPR conditions: temperature, 4.2 K; microwave power, 35 dB (63 μW); microwave frequency, 9.44 GHz; modulation amplitude, 22 G; gain $2 \cdot 10^5$. $Q_A^- Fe^{2+}$ (c and d) were recorded in the dark with EPR instrument settings as for the triplet except that the temperature was 4.7 K, microwave power, 8 dB (32 mW) and the gain $2 \cdot 10^4$.

continuous illumination was provided at liquid helium temperature (Fig. 1a). Room temperature illumination of dithionite reduced PS II results in the trapping of Ph^- [24] and the loss of the $Q_A^- Fe^{2+}$ signal [15,16] presumably due to its double reduction. The trapped Ph^- exhibits a 13 G wide free radical EPR signal at $g = 2.0033$ [24]. Dark adaptation of such a sample results in the slow detrapping of the Ph^- radical, as this highly reducing species ($E_m \approx -600$ mV [25,26]) equilibrates with the environment (not shown). The $Q_A^- Fe^{2+}$ EPR signal is not regenerated by this treatment (Fig. 1d). Illumination at 4 K of PS II pretreated in this way results in the formation on an easily detectable triplet EPR signal (Fig. 1b) with the polarization pattern typical of its formation by radical-pair recombination (see [27]).

Fig. 1 shows that when the $Q_A^- Fe^{2+}$ signal is diminished, the triplet signal greatly increases. To determine whether these two effects are linked or whether one or both are due to non-specific photodamage, other methods of double reducing the quinone were looked for. Experiments were performed in which PS II membranes were incubated with sodium dithionite in darkness at room temperature. At various times during the incubation, samples were frozen to 4 K and the photoinduced triplet and $Q_A^- Fe^{2+}$ signals were recorded. A decrease occurred in the $Q_A^- Fe^{2+}$ signal by approx. 50% in 5.5 h; the photoinduced triplet signal increased with a similar time dependence (not shown). Due to the very long incubation times required, we looked for a redox mediator that would accelerate the reduction process. Fig. 2a shows that, in the presence of benzyl viologen, the $Q_A^- Fe^{2+}$ disappeared upon dark incubation with dithionite with a $t_{1/2}$ of approx. 1 h 20 min. The triplet signal amplitude increased with a similar time dependence. The close correlation between the loss of the $Q_A^- Fe^{2+}$ signal and the appearance of the photoinduced triplet is clearly shown in Fig. 2b.

The behaviour of the $Q_A^- Fe^{2+}$ signal and of the photoinduced triplet signal were investigated under the controlled conditions of redox titrations. Decreasing the ambient redox potential inevitably results in the chemical reduction and consequent release of the Mn ions associated with the oxygen evolving activity of PS II. The material used in the redox titrations was Tris-washed to remove the Mn and prevent any slow evolution of the state of the electron donor side of PS II during the course of the experiments.

Fig. 3 shows that the first reduction of $Q_A Fe^{2+}$ forming $Q_A^- Fe^{2+}$ (measured in the presence of sodium formate as the signal at $g = 1.85$ [23]) occurs with an $E_m \approx -16$ mV (obtained by fitting with an $n = 1$ Nernst curve). The signal from $Q_A^- Fe^{2+}$ disappears at redox potentials lower than approx. -350 mV. The ap-

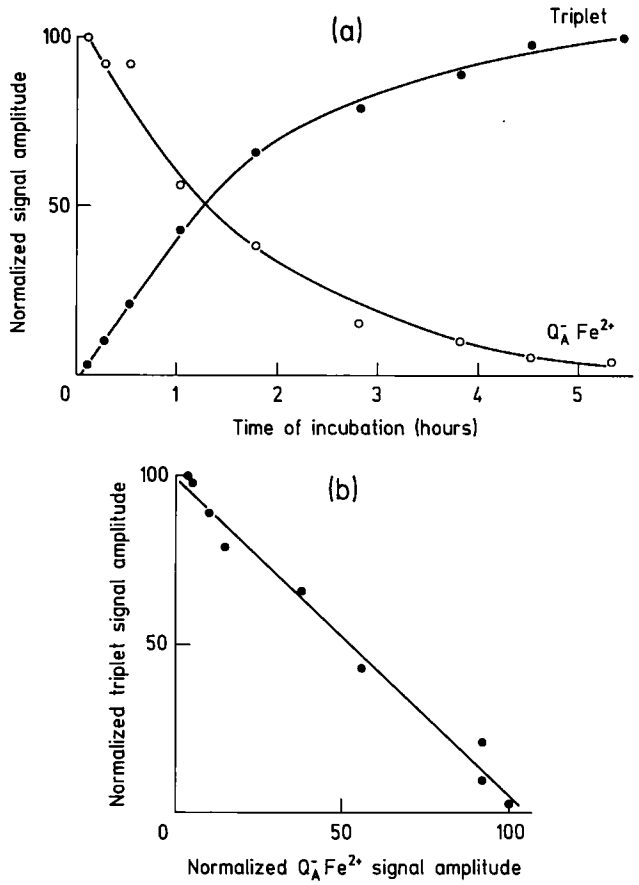

Fig. 2. Dark incubation under reducing conditions in the presence of benzyl viologen. Sample conditions were the same as in Fig. 1 except that no $CaCl_2$ was present, the EDTA concentration was 0.5 mM and 34 μM benzyl viologen was also present. (a) The amplitude of the triplet EPR signal (●) and the $Q_A^- Fe^{2+}$ signal (○) as a function of dark incubation time. (b) The amplitude of the triplet signal plotted as a function of the amplitude of the $Q_A^- Fe^{2+}$ signal in the same experiment as (a). The signal amplitudes were measured as follows: $Q_A^- Fe^{2+}$, the height of the signal measured between the peak maximum at $g \approx 1.85$ and the trough at $g \approx 1.7$; triplet, as the height of the lowest field line. EPR conditions were as described in Fig. 1 except that the gain was $8 \cdot 10^4$ and the modulation amplitude was 25 G.

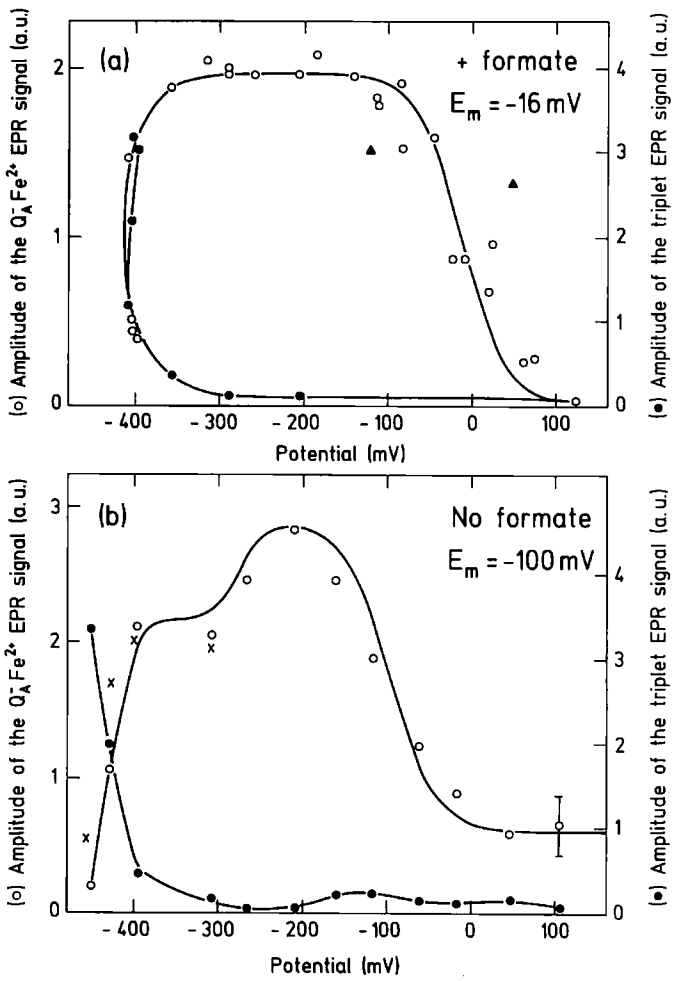

Fig. 3. Redox titrations at pH 7.0 of Tris-washed PS II enriched thylakoid membranes. The amplitude of the triplet (●) and $Q_A^- Fe^{2+}$ (○) spectra are plotted versus ambient potential. The amplitude of the triplet when reduced samples were reoxidized (▲) are also shown. (a) redox titrations in the presence of 200 mM formate. (b) redox titrations without addition of formate. EPR conditions were as in Fig. 1, except that $Q_A^- Fe^{2+}$ in the absence of formate was recorded using 32 G modulation amplitude and 6 dB (40 μW). An estimate of the error in recording $Q_A^- Fe^{2+}$ arising from the poor signal to noise in the absence of formate is shown by the error bars for a point in (b). The amplitude of the split Ph^- EPR signal (×) induced by a period of illumination at 200 K monitored during the redox titrations is also shown in (b) EPR conditions were as for $Q_A^- Fe^{2+}$ in Fig. 1 except that the modulation amplitude was 16 G.

pearance of the photoinduced triplet corresponds closely with the loss of the $Q_A^- Fe^{2+}$ signal. Once this redox transition had occurred, titrations in the oxidizing direction resulted in no reappearance of the $Q_A^- Fe^{2+}$ signal and only a minor (approx. 30%) decrease in the triplet signal amplitude (Fig. 3a, solid triangles). This redox transition does not follow a Nernst curve, the curve drawn is hand-fitted to the points, the position of which varied with the sampling frequency.

As a control for the possible involvement of formate in the redox characteristics of $Q_A Fe^{2+}$, experiments were performed in the absence of formate (Fig. 3b). As expected, the $Q_A^- Fe^{2+}$ signal is much smaller [23] and it is dominated by a signal centered at $g = 1.9$ at this pH [16]. Due to the decrease in signal amplitude the data are noisier. However, it seems that the first reduction step of $Q_A Fe^{2+}$ reduction occurs with an E_m value (−100 mV) lower than that in the presence of formate *. In addition, a slight decrease in $Q_A^- Fe^{2+}$ signal am-

* The difference in the $E_{m,7.0}$ for $Q_A Fe^{2+}/Q_A^- Fe^{2+}$ in Fig. 3a and b may indicate a formate-induced negative shift. Vermaas and Govindjee [42], however, reported that addition of bicarbonate to a formate-treated thylakoid did not induce a shift in the E_m ($E_{m,6.5}$, −145 mV). This apparent discrepancy may be due to the different comparisons made (i.e., untreated vs. formate-treated here, formate-treated vs. formate-treated plus addition of bicarbonate [42]), but also might by due to other differences in experimental conditions (pH, material, etc.).

plitude occurs between −200 mV and −300 mV. The origin of this slight decrease is not clear; however, it does not seem to be associated with an increase in the triplet signal. Despite these minor differences, the major decrease in the $Q_A^-Fe^{2+}$ occurs at low potential and this corresponds to the appearance of the photoinduced triplet signal, just as observed in the presence of formate.

In response to a reviewer's comment, in Fig. 3b we also show the amplitude of the split Ph^- signal (crosses) over the potential range in which the $Q_A^-Fe^{2+}$ signal disappears. The signal is generated by illumination at 200 K and arises from an interaction between Ph^+ and $Q_A^-Fe^{2+}$ [24]. The signal is lost with the same potential dependence shown for the loss of $Q_A^-Fe^{2+}$ and the appearance of the triplet. This result is in apparent contrast to the report that the split Ph^- signal decreases at potentials lower than those required for appearance of the triplet signal [6]. We suggest that the observations in Ref. 6 might be due to the fact that the data for the triplet and the split Ph^- were recorded in different titration experiments. Variations in the apparent redox dependencies for this transition are likely to be obtained from titration to titration because the redox event being monitored is slow and irreversible. Under such conditions, the form of the 'redox curve' is more dependent on time (i.e., sampling frequency) than on the ambient redox potential.

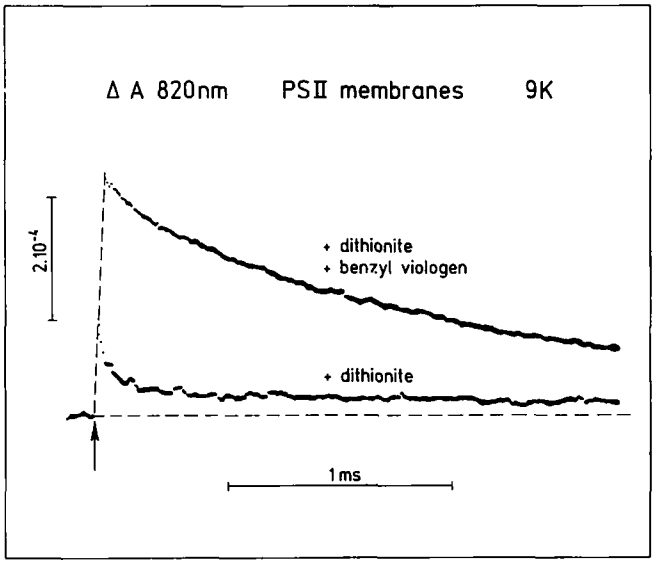

Fig. 4. Flash-induced absorption changes at 820 nm at 9 K in sodium dithionite-reduced PS II membranes incubated in the presence and absence of benzyl viologen (34 μM). The chlorophyll concentration was 110 μg per ml in a buffer comprising 50% ethylene glycol/40 mM sodium dithionite/200 mM sodium formate/82 mM Mops (pH 7.0)/8.4 mM NaCl/4.2 mM $MgCl_2$/1 mM EDTA. The sample incubated (about 3 h) in benzyl viologen contained 1.4 μM of this mediator in the cuvette. The sample in the absence of benzyl viologen was incubated for about 1 h. The cuvette thickness was 1.1 mm. The traces are the average of 32 flashes.

To verify that the amplitude changes of the EPR triplet signal are a direct reflection of the triplet yield, experiments were performed using flash absorption kinetic spectrophotometry. The reaction-centre triplet yield was measured by its absorption increase at 820 nm which decays with a $t_{1/2}$ of approx. 0.9 ms at liquid helium temperature [28,29]. Samples were treated with sodium dithionite in the presence or absence of benzyl viologen for different times of incubation in darkness. The EPR properties of the samples were monitored prior to their dilution and use in the absorption experiments. Fig. 4 shows the results of such an experiment. A small change is seen at 820 nm in the sample incubated in dithionite alone. Most of this small change decays rapidly ($t_{1/2} \approx 50$ μs). The origin of this phase is unknown. A smaller longer-lived phase may represent a small amount triplet formation as predicted from the EPR measurement (yield < 20%). In the sample treated with benzyl viologen, a much bigger absorption change is seen. This decays with a $t_{1/2}$ of approx. 1.1 ms and is attributed to the reaction centre triplet. Using the approximate absorption coefficient for the triplet of 3800 $M^{-1} \cdot cm^{-1}$ [21], it can be calculated that the triplet yield in the sample treated with benzyl viologen is close to 100%, while that with dithionite alone is less than 20%.

Although these yields are rather uncertain due to the large uncertainty in the P680 triplet absorption coefficient, their relative values correspond well with the amplitude of the triplet signal observed by EPR using the same samples.

Discussion

The earlier observation that the reduction of a component at low potential (about −350 mV) must occur before the triplet can be generated [6] is verified by the current work. Here, however, we show that the ability to photoinduce the triplet correlates with the loss of the $Q_A^-Fe^{2+}$ signal. Thus, there is no need to invoke the presence of a new redox component between Ph and Q_A. Instead, the redox transition responsible is presumed to be the second reduction of the quinone, forming the fully reduced quinol.

Direct evidence for formation of the quinol is lacking; the argument relies heavily on precedence in the bacterial reaction centre [12–14] and on reasonable expectations of quinone chemistry under photochemical and chemical reduction conditions. In the bacterial system the quasi-reversibility of the loss of $Q_A^-Fe^{2+}$ provided confidence in this explanation. In PS II, however, at least in the titration work reported here, we were unable to see such reversibility. The suggested loss of the QH_2 from the site (see Introduction) could be followed in PS II by an irreversible change in the Q_A site which prevents rebinding of PQ. This scenario is

not unreasonable, since it is known that the Q_A site is labile in the absence of the quinone [30] (see also Ref. 31). It is of note that a small degree of reversibility of $Q_A^-Fe^{2+}$ double-reduction in PS II was reported earlier in photoreduced samples which were rapidly reoxidized [15].

The nature of the effect by which $Q_A^-Fe^{2+}$ prevents triplet formation (or by which its double reduction induces triplet formation) is not clear. However, some of the possible explanations are considered below. (1) A direct effect of $Q_A^-Fe^{2+}$ on the P^+Ph^- radical pair could occur via an electrostatic interaction between Q_A^- and Ph^-. This would make the $P680^+Ph^-$ radical pair a higher energy state, displacing the equilibrium, $^*P680Ph \leftrightarrow P680^+Ph^-$, to the left. These effects could result in the radical pair having a smaller yield and possibly a shorter lifetime, hence the triplet yield would be greatly diminished. (2) A direct magnetic effect of $Q_A^-Fe^{2+}$ on the radical pair could influence spin dephasing, favouring singlet radical-pair recombination. Both of these direct effects would be removed by the second reduction of Q_A. The electrostatic effect could be lost due to the protonation of the fully reduced form and its probable simultaneous detachment from the Q_A site. The magnetic effect would be lost because the quinol is diamagnetic. (3) Indirect effects, due to secondary events associated with the second reduction of Q_A, could influence $P680^+Ph^-$ radical pair yield, lifetime and back-reaction pathway. Such secondary events include, for example, protonations, conformational changes perhaps associated with debinding of the quinol from the Q_A site.

From considerations of the literature we favour the first of these explanations. A direct electrostatic effect of Q_A^- on the $P680^+Ph^-$ radical pair has been invoked recently to explain ps/ns fluorescence and absorption data [32,33] (see also Ref. 34). A lower yield of $P680^+Ph^-$ radical-pair formation was observed in closed PS II reaction centres (i.e., when Q_A^- is present) compared to open PS II reaction centres (i.e., when Q_A is present). An interaction between Q_A^- and $P680^+Ph^-$ was estimated to increase the free energy of the radical pair by 50 mV [32]. It is reasonable to propose that when Q_A is double-reduced or absent, the free energy of the $P680^+Ph^-$ radical pair could resemble that in open centres. Fig. 5 shows a simplified schematic representation of the influence of the redox state of Q_A on the free energy level of $P680^+Ph^-$ and is an extension of the model of Schatz et al. [32].

It is also worth pointing out that the 'jammed' state is likely to be a lower fluorescent state that the closed state. Experiments correlating the EPR changes with fluorescence changes are required before the extent of the quenching can be estimated.

The kinetics of $P680^+Ph^-$ radical pair formation in PS II membranes have already been studied under

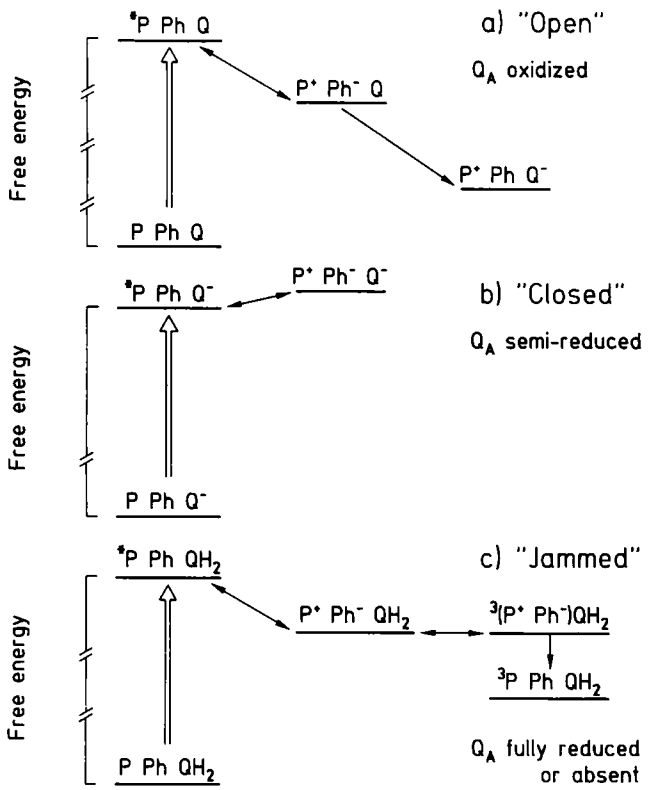

Fig. 5. Simplified schemes of the free energy levels of the states involved in the primary photochemistry of PS II with different redox states of Q_A (Q). The three states (a) open, (b) closed and (c) 'jammed', correspond to Q_A being oxidized, semireduced and fully reduced (or absent), respectively. Absorption of a photon of light in the (a) open and (c) 'jammed' conditions results in a high quantum yield of charge separation. In case (a) this leads to charge stabilization by electron transfer from Ph^+ to Q_A. In case (c) this leads to triplet formation. Absorption of light in the (b) closed condition gives rise to a very low yield of charge separation and the energy is wasted as prompt fluorescence. In addition, the antenna size is thought to influence the yield of charge separation [32,35,36]. See the text for further details.

conditions which correspond to those of Fig. 1, i.e., the closed and 'jammed' states in Fig. 5 [34]. The phenomena observed could be interpreted in two ways: either the radical pair lifetime increased from less than 500 ps to 3 ns or the quantum yield of charge separation greatly increased upon preillumination and dark adaptation, a treatment now known to result in the loss of $Q_A^-Fe^{2+}$. Both of these explanations would be consistent with our observation of the appearance of the photoinduced triplet in the preilluminated sample. The explanation involving an increase in the quantum yield fits better with the scheme in Fig. 5 and with the models of Schatz et al. [32] and Schlodder and Brettel [33].

In purple bacteria, schemes similar to those in Fig. 5 can be drawn; however, in all three states the free energy level of P^+BPh^- would be low enough to result in a 100% quantum yield of charge separation. What then could be the origin of the difference in the energet-

ics (and thus the photochemistry) seen when comparing PS II and purple bacteria under conditions where Q_A is present?

The first factor to be considered is the postulated electrostatic interaction between Q_A^- and $P680^+Ph^-$. Even if it is assumed that the chromophores occupy identical relative positions in both reaction centres, differences in amino acid residues in the region of the Ph and Q_A could result in a local dielectric that favours the electrostatic interactions between the two negatively charged species in PS II. Very recently, exactly such a diminished dielectric in PS II was postulated from photovoltage measurements [37].

Secondly, the intrinsic redox potentials of the components in PS II could make the free energy difference between $^*P$ and $P680^+Ph^-$ in the open state smaller than the corresponding value in the purple bacteria reaction centre. Thus the influence of Q^- on $P680^+Ph^-$ could be more marked in PS II, even if the electrostatic interaction in both kinds of reaction centre were the same.

Thirdly, the presence of a large number of antenna chlorophylls in PS II, many of which have long-wavelength maxima close in energy to P680, makes P680 a very 'shallow trap' [32,35,36]. An equilibrium distribution towards the excited state rather than the radical pair is favoured by increasing the size of the antenna: $^*[Chl]_n$ P680Ph ↔ $[Chl]_n$ *P680Ph ↔ $[Chl]_nP680^+Ph^-$.

A combination of a larger electrostatic effect of Q_A^- on $P680^+Ph^-$ in PS II and the influence of the antenna on the yield of charge separation may best describe the situation observed in PS II. Studies of the $P680^+Ph^-$ yield and lifetime have been performed using a range of PS II preparations with varying antenna sizes. Increases in the radical-pair lifetime and yield were observed as the antenna size decreased [38], in accordance with the shallow trap theory [35]. However, the correlation was far from perfect and it was concluded that antenna size was not the only factor influencing the equilibrium. It now seems likely that the additional factor is the presence of $Q_A^-Fe^{2+}$. It is also clear that, at least under our conditions, this is the dominant factor influencing the energetics of the radical pair.

It was reported earlier that harsh detergent treatments result in marked increases in the amplitude of the triplet EPR signal [28]. It now seems likely that this is due to a detergent-induced loss of the $Q_A^-Fe^{2+}$ complex and also perhaps to the diminution of the functional antenna size. A survey of several different PS II preparations seems to confirm the existence of an inverse relationship between the triplet state and the $Q_A^-Fe^{2+}$ signal. PS II-enriched membranes show little or no triplet under conditions in which a large $Q_A^-Fe^{2+}$ signal is observable (e.g., Fig. 1). In the smallest PS II preparations, $Q_A^-Fe^{2+}$ is lacking and the triplet is easily seen [39]. A range of different core preparations, which can be classed between these two extremes, show intermediate properties, i.e., variable amounts of triplet [4,8] and $Q_A^-Fe^{2+}$ (e.g., Ref. 8 and A.W.R., unpublished data). According to the current work and the literature discussed above, the presence of the triplet in such preparations could reflect two additive effects: the degree of $Q_A^-Fe^{2+}$ destroyed by the detergent treatment and the size of the functional antenna. The relative influence of each one of these effects is difficult to judge. In this regard, it will be extremely useful to look at the triplet yield in D1/D2 preparations which still maintain Q_A when (if) such a preparation is isolated.

It has been recently reported that the dithionite-reducable $Q_A^-Fe^{2+}$ signal is lost simultaneously with PS II electron transfer during photoinhibition of PS II with strong light [40]. Since Ph^+ photoaccumulation has been demonstrated under strong light in the absence of chemical reductants [41], it was predictable that Q_AFe^{2+} double-reduction should occur under these conditions. We can now propose that the changes in primary photochemistry due to $Q_A^-Fe^{2+}$ double-reduction which are reported here (i.e., increased triplet yield, probably increased $P680^+Ph^-$ yield and possibly lifetime) are likely to be found in photoinhibited material. In addition, since we have observed that the double-reduction of Q_A is an irreversible process, we suggest that this event could be the primary lesion which results in an irreversible electron transfer block in PS II during photoinhibition.

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