Biochimica et Biophysica Acta, 1101 (1992) 311–320

BBABIO 43640

Reconstitution and exchange of quinones in the A_1 site of Photosystem I. An electron spin polarization electron paramagnetic resonance study

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(Received 24 December 1991)

Key words: Photosystem I; EPR; Electron spin polarization; Vitamin K_1; Phylloquinone; A_1 acceptor

The electron spin polarized (ESP) electron paramagnetic resonance (EPR) signal observed in spinach Photosystem I (PS I) particles was examined in preparations depleted of vitamin K_1 by solvent extraction, followed by reconstitution with a series of quinones and quinone analogues. The ESP EPR signal was previously attributed to a radical pair that included vitamin K_1^- (Rustandi, R.R., et al. (1990) Biochemistry 29, 8030–8032) and, in addition, vitamin K_1 was assigned as the secondary acceptor A_1 in PS I (Snyder, S.W., et al. (1991) Proc. Natl. Acad. Sci. USA, 88, 9895–9896). The ESP EPR signal was observed in untreated PS I preparations, was not detected in the solvent-extracted PS I samples and was restored upon reconstitution using certain quinones. The ability to restore the ESP EPR signal was dependent upon two properties of the reconstituted acceptor. First, the solution reduction potential of the reconstituted acceptor must be more positive than about −750 mV where the solution reduction potential of vitamin K_1 is −710 mV. Second, the structure of the reconstituted acceptor requires either a minimum of two aromatic rings (i.e., naphthoquinone) or a benzoquinone (or larger) derivative substituted with an alkyl tail. A model was developed to describe both the requirements for electron transfer to A_1 and also previous results for electron transfer from A_1^- to the iron-sulfur centers (Biggins, J. (1990) Biochemistry 29, 7259–7264). When untreated PS I preparations were incubated with perdeuterated vitamin K_1 (DK_1) the endogenous K_1 was observed to exchange with DK_1. The replacement rate was strongly dependent upon temperature (h–days at 4°C, min at 37°C) and upon illumination (min). Naphthoquinones lacking a long alkyl tail were unable to exchange with endogenous vitamin K_1. Although no known physiological role exists for vitamin K_1 ejection from its A_1 binding site, the quinone appears to be somewhat labile. Direct exchange of vitamin K_1 with exogenously supplied quinones indicates that the PS I A_1 site might be a target for the design of new herbicides in green plants.

Introduction

The Photosystem I (PS I) reaction center (RC) in green plants, algae and cyanobacteria, is a membrane-bound, protein/pigment complex which facilitates the photoinduced electron transfer from plastocyanin to ferredoxin. In PS I the stabilization of charge separation is achieved by successive electron transfer steps through a series of redox centers that ultimately lead to $NADP^+$ reduction (for reviews see Refs. 1–4). An atomic level crystal structure is unavailable for PS I and, therefore, we seek alternative approaches to determining the structure and function of PS I. (The reaction center structures of two purple photosynthetic bacteria have been determined by X-ray crystallography [5–8]). The chemical identity of each of the PS I electron donors and acceptors has not been unequivocally assigned. The PS I electron transfer chain is generally believed to be $P_{700}A_0A_1F_xF_{a/b}$, where the primary donor, P_{700}, is a chlorophyll *a* dimer; the acceptor A_0 is a chlorophyll *a* monomer; A_1 is the substituted naphthoquinone, vitamin K_1; F_x is an inter-polypeptide iron-sulfur [4Fe-4S] center and F_a and F_b are the terminal iron-sulfur [4Fe-4S] centers (P_{430}). We refer to all of the iron-containing acceptors following A_1 collectively as the 'FeS centers'.

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Abbreviations: ESP, electron spin polarization; EPR, electron paramagnetic resonance; PS I, Photosystem I; K_1, vitamin K_1 (phylloquinone); PK_1, protonated vitamin K_1; DK_1, perdeuterated vitamin K_1; RC, reaction center.

There have been numerous investigations of electron transfer kinetics in PS I [1,2]. Electron transfer can be blocked at various sites along the pathway by either chemical reduction or biochemical extraction, allowing back electron transfer from the previous acceptor to P_{700}^+. Forward reaction rates and recombination rates between the reduced acceptors and P_{700}^+ have been determined at both cryogenic and room temperatures [1,2].

Vitamin K_1 or phylloquinone (K_1) is the only quinone found in stoichiometric quantities in PS I (approximately two molecules co-purify with the reaction center [9–12]) and its role as the secondary acceptor A_1 has been assigned recently [13]. The function of K_1, and its identity as A_1 in PS I have been investigated by a variety optical [11,14–28] and electron paramagnetic resonance (EPR) techniques [11,13,19,29–36]. On the other hand, several reports have offered conflicting evidence against the assignment of K_1 as the acceptor A_1 in PS I [11,19,32]. The putative A_1^- EPR signal was observed from PS I samples in which K_1 was either destroyed in situ by ultraviolet light irradiation [11,19] or selectively substituted with deuterium [32]. These results appear contradictory with the assignment of K_1 as a functional intermediate in the electron transfer pathway, but recent results suggest that the EPR signal attributed to A_1^- may be due to a different photoaccumulated signal [27].

Millisecond transient optical measurements were employed to monitor electron transfer to the terminal FeS centers (identified as P_{430} turnover) [21,22]. In PS I D144-particles, P_{430} turnover was interrupted by extraction of K_1 with a hexane/methanol solvent, and could be restored by chemical reconstitution of K_1. A number of different quinones were tested as replacements for restoration of electron transfer to the FeS centers. Although all of the quinones tested acted as electron acceptors from A_0^-, only three naphthoquinone derivatives restored P_{430} turnover. A long alkyl tail at the 3 position and a methyl group at the 2 position were required to restore P_{430} turnover [22]. These results are at variance with the position of Iwaki and Itoh who claim less specificity in the quinone intermediate for electron transfer to the FeS centers [23–26,37,38]. Perhaps this discrepancy arises from either the different solvents used for the quinone extraction procedure (ether/water versus hexane/methanol) or the different approach employed to optically monitor electron transfer.

A characteristic electron spin polarized (ESP) EPR signal was first observed in PS I a number of years ago [29–31,33,34,39–43]. We have demonstrated that in spinach PS I samples that have undergone the extraction/reconstitution procedure, this ESP EPR signal arises from a radical pair composed of P_{700}^+ and K_1^- [31]. In the extracted PS I sample, at approximately the same optical density as the control sample, we did not observe any ESP EPR signal [31]. By monitoring the ESP EPR signal under chemically reducing conditions that are considered to doubly reduce A_1 to A_1^{2-} [27,28], we went on further to attribute the ESP EPR signal to the P_{700}^+ A_1^- radical pair [13]. From this work we were able to unequivocally assign K_1 as the secondary acceptor A_1.

Decay of the P_{700}^+ A_1^- radical pair by electron transfer to the FeS centers can be followed by room temperature time-resolved EPR experiments (TR-EPR) [33,34,39,40,44,45]. When TR-EPR experiments were combined with the extraction/reconstitution protocol, a greater specificity in the replacement quinone was observed for electron transfer from the reduced quinone to the FeS centers than for reduction of the quinone [22,34].

In this report we investigate the dependence of the low temperature ESP EPR signal of PS I preparations upon reconstitution with a series of 1,4-benzoquinones, 1,4-naphthoquinones, 9,10-anthraquinones, and a series of quinone analogues. The ESP EPR signal was monitored either by a light-modulation, phase-sensitive detection, or by a pulsed-laser, direct-detection technique. The EPR results are compared to the previous transient optical results on P_{430} turnover. We discuss both the requirements for electron transfer from A_0^- to the quinone and the more stringent requirements for electron transfer from the reduced quinone to the FeS centers.

We also report on a new technique in which the endogenous K_1 in PS I is exchanged, or replaced, by incubation of the PS I with an exogenous quinone without prior K_1 extraction. Since the PS I preparation was not extracted with organic solvents, the preparation more closely resembles native PS I. From these exchange experiments we demonstrate that the replacement quinones reconstitute the A_1 acceptor site in PS I.

Materials and Methods

Spinach PS I D144-particles were isolated according to the procedures of Anderson and Boardman [46] and lyophilized. For quinone-depleted samples, quinones were extracted using a 0.3% methanol/hexane solvent mixture, by procedures described previously [21,22]. The untreated (control) and extracted PS I particles were rehydrated in 50 mM Tris buffer (pH 7.5) with 0.2% Triton X-100 and 2 mM sodium ascorbate and centrifuged to remove insoluble material [20–22,31]. The samples were adjusted to a final absorbance of 15 A.U./cm at the absorption maximum near 670 nm. The chlorophyll concentration was about 260 μg/ml in the control/exchange samples and 300 μg/ml in the extracted/reconstituted samples [22,47]. In a similar

PS I preparation, P_{700} concentration was estimated at 1 μM for the control/exchange samples and about 50% of the antenna chlorophyll was lost for the extracted/reconstituted samples [22,47].

The quinones and analogues were obtained from a variety of sources, dissolved in ethanol (1 mM) and added directly to the PS I preparation. Several quinones were dissolved in ethanol and checked for EPR response under illumination conditions employed in the ESP EPR experiment as controls, and no ESP EPR signals were observed. Protonated K_1 (PK_1) was obtained from Aldrich and its purity was checked by desorption mass spectroscopy. Perdeuterated K_1 (DK_1) was extracted from the cyanobacterium *Synechoccus lividus* (99.6% deuterated), purified by HPLC and its purity was verified by absorption spectroscopy and desorption mass spectroscopy. The following quinones and quinone analogues were obtained from Aldrich and used as received: 1,4-naphthoquinone (NQ), 2,3-dichloro-1,4-NQ (Cl_2NQ), 2-ethyl-1,4-NQ (Et-NQ), 2-methyl-1,4-NQ (PK_3), 2,3-dimethyl-1,4-NQ (Me_2NQ), 1,4-benzoquinone (BQ), duroquinone (DQ), 9,10-anthraquinone (AQ), 1-nitro-9,10-AQ (NO_2-AQ), 2-methyl-9,10-AQ (Me-AQ), 1-amino-9,10-AQ (NH_2-AQ), chloroanil, fluorenone, and anthrone. The ubiquinones (UQ-)0,6,9,10 and 2-methyl-3-[isoprenyl]$_4$-1,4-NQ (MQ-4) were obtained from Sigma and used as received. The following quinones were obtained from Alfred Bader and checked for purity by thin layer chromatography (TLC): 2-ethylthio-3-methyl-NQ (EtS-Me-NQ),2-octadecylamino-1,4-NQ($NC_{18}NQ$), 2-tetradecylamino-1,4-NQ($NC_{14}NQ$),2,3-dichloro-6-nitro-NQ-(Cl_2NO_2NQ),2-chloro-3-(4-fluoroanilino)-1,4-NQ(Cl-FAn-NQ),2-chloro-3-(3-trifluoromethyl-anilino)-NQ (Cl-CF_3An-NQ), 1,4-NQ-sulfonic acid, potassium salt (NQ-SO_3H^-), and 2-chloro-3-oleylamino-1,4-NQ (Cl-OA-NQ). Cl-OA-NQ was purified by silica gel column chromatography (chloroform mobile phase) and was re-checked for purity by TLC and desorption mass spectroscopy. Stigmatellin and 5-n-undecyl-6-hydroxy-4,7-dioxobenzothiazole (UHDBT) were gifts from D.E. Robertson (University of Pennsylvania, Philadelphia). Perdeuterated 2-methyl-1,4-NQ (DK_3) and 2-ethyl-3-methyl-NQ (Et-Me-NQ) were gifts from C. Bender (Albert Einstein College, New York). The quinone 2-cyclopropyl-5-butyl-benzoquinone (CP-Bu-BQ) was a gift from D. Lynn (University of Chicago, Chicago). The quinones 2-methyl-3-decyl-1,4-NQ (Me-De-NQ) and 2-decyl-1,4-NQ (De-NQ) were gifts from P. Rich (Glynn Research Institute, Bodmin, UK).

Quinone-depleted PS I samples were reconstituted by addition of the desired quinone directly to the PS I preparation (50 μM final concentration). Quinones were incubated with the PS I samples overnight at 4°C in the dark. All samples were dark-adapted at 4°C for 10 min prior to freezing in liquid nitrogen. Quinone exchange experiments were conducted in a similar fashion to the extraction/reconstitution experiments except that the desired quinone was added directly to the 'untreated' PS I preparations. In the exchange experiments the incubation time ranged from 10 min (minimum time required to dark adapt the samples) to 48 h. The quinone concentrations varied between 10 μM and 250 μM and the incubation temperature was adjusted between 4°C and 37°C. Photolyzed samples were illuminated in an ice bath using a focused 350 watt projector lamp for 10–30 min.

EPR X-band measurements were performed on a Varian E-9 Spectrometer equipped with an Air Products low-temperature accessory. A microwave frequency in the range 9.1–9.3 GHz, a magnetic field center of 320–330 mT and a scan range of 10 mT were employed for all experiments. Zeeman field modulation was performed at a frequency of 100 KHz and 0.125 mT amplitude (instrument settings) and, therefore, the spectra are reported as the first derivative of the absorption mode. Preliminary scans were obtained on the dark samples to check for spurious signals. The g-factors were calibrated by comparison to a powder 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical sample ($g = 2.0037 \pm 0.0002$) and a Mn^{2+}/SrO sample. All spectra were recorded at a temperature of 13 ± 1 K.

Samples were excited with an ILC 300 W visible xenon arc lamp filtered using a 5 cm pathlength aqueous sodium nitrite solution to remove both ultraviolet light (400 nm cutoff) and infrared light. In light-modulation EPR (LM-EPR) experiments, the light intensity was sine wave-modulated by a Wavetek Model 180 function generator at 500 Hz and ESP EPR signals were collected with a phase-sensitive detection system. Microwave power was maintained between 0.2–0.5 mW. The signal from the Varian EPR console (time constant out) was amplified using a PAR EG&G 5208 dual channel lock-in amplifier referenced to the modulated light source [48,49]. After demodulation, the amplified signal was digitized and averaged for 12 min on an IBM AT computer. Phase angles were adjusted to maximize the ESP EPR signals and spectra were collected 90° out-of-phase to check that the signal was collected at the phase maximum.

In direct-detection, time-resolved EPR (TR-EPR) experiments, samples were excited by a Lumonics dye laser (585 nm, 5 mJ/pulse) pumped by Questek 2400 excimer laser (308 nm) operated at 20 Hz. The signal was taken directly from the Varian EPR bridge (1 mW power), amplified, and integrated by a dual-channel (164) PAR EG&G 162 boxcar averager, that was triggered by photodetection of the laser [50]. TR-EPR signals were collected at a time delay of 1 μs. In the direct-detection experiments no field modulation was applied and, therefore, spectra were collected in the non-derivative absorption mode.

Results and Discussion

Continuous illumination

Photoinduced EPR signals of P_{700}^{+} obtained by continuous illumination of the control, extracted, and reconstituted PS I samples were all very similar with *g*-factors around 2.0025 and linewidths of about 0.75 mT. All signals were found to be about 20% reversible. Signals from the terminal FeS centers (F_a^- and F_b^-) were also observed in control and extracted PS I samples upon continuous illumination at cryogenic temperatures, in agreement with previous work [20]. In comparison, at room temperature complete quinone depletion blocks reduction of the FeS centers, but K_1 reconstitution restores this electron transfer [21,22]. This discrepancy was attributed to a structural reorganization in PS I at low temperature that permits electron transfer to proceed efficiently from A_0 to the FeS centers in the

TABLE I

Reconstitution of quinones in hexane / methanol-extracted PS I

Reconstituted quinone	ESP EPR signal	ESP EPR comments [a]	P_{430} turnover [b]	Reduction potential (mV) [c]
Control	+	medium	+	
Extracted	–	no signal	–	
PK_1	+	medium	+	−710 [d]
DK_1	+	strong, deuterated		
PK_3	+	medium	–	−650 [e]
DK_3	+	strong, deuterated		
NQ	+	strong	–	−581 [e]
Et-NQ	+	medium		−670 [f]
De-NQ	+	medium	–	−680 [f]
Me_2NQ	+	weak	–	−746 [e]
Et-Me-NQ	+	medium		−746 [f]
Me-De-NQ	+	medium	+	−730 [e]
MQ-4	+	medium	+	−709 [e]
Cl_2NQ	+	strong, *g*-factor shift		−300 [d]
Cl-OA-NQ	+	weak		−660 [g]
EtS-Me-NQ	+	weak		−700 [f]
$NC_{11}NQ$	–	no signal		−810 [f]
$NC_{18}NQ$	–	no signal		−810 [f]
Cl_2NO_2NQ	–	no signal		
Cl-FAn-NQ	+	strong		
Cl-CF_3An-NQ	+	strong		
NQ-SO_3H^-	+	strong		−510 [f]
BQ	–	no signal		−401 [e]
DQ	–	no signal	–	−750 [h]
UQ-0	+	medium		−645 [e]
UQ-6	+	medium		−600 [e]
UQ-9	+	medium		−600 [e]
UQ-10	+	medium		−600 [e]
2-CP,5-Bu-BQ	+	weak		−755 [f]
Chloroanil	–	no signal		−80 [f]
AQ	–	no signal	–	−830 [h]
NO_2-AQ	+	medium		−590 [d]
Me-AQ	–	no signal		−850 [h]
NH_4-AQ	–	no signal		−940 [h]
Anthrone	–	no signal		
Fluorenone	–	no signal		
Stigmatellin	–	no signal		
UHDBT	–	no signal		

[a] Signal-to-noise ratios in spectra from control and PK_1-reconstituted samples were estimated at 10. Therefore, our lower limits of signal detection are no more than about 10% of the signal observed in control or PK_1-reconstituted samples.

[b] P_{430} turnover is described in the text. (From Biggins, J. (1990) Biochemistry 29, 7259–7264).

[c] Values are for DMF solution and may change in the photosynthetic preparation.

[d] Iwaki, M. and Itoh, S. (1989) FEBS Lett. 256, 11–16.

[e] Prince, R.C., Dutton, P.L. and Bruce, J.M. (1983) FEBS Lett. 160, 273–276.

[f] Estimated.

[g] Wa Lewski, M.R., unpublished data.

[h] Woodbury, N.W., Parson, W.W., Gunner, M.R., Prince, R.C. and Dutton, P.L. (1981) Biochim. Biophys. Acta 851, 6–22.

absence of A_1. An alternative explanation is that low-temperature electron transfer from A_0^- to the FeS centers may be able to directly compete with other A_0^--deactivation pathways that rapidly deplete the A_0^- population at room temperature.

Reconstitution of quinones and analogues in PS I

Summarizing the preliminary report, for the control and PK_1-reconstituted samples [31], we observed the 'normal' ESP EPR signal with the usual emission/absorption/emission (E/A/E) pattern and similar *g*-factors [29–31,33,34,40–43]. Reconstitution with DK_1 restored the ESP EPR signal but with a linewidth narrowing (by a factor of about 1.6) in a portion of the ESP EPR signal [31]. The ESP EPR exhibited an E/A/E pattern but an additional weak absorption signal appeared at low field. Low signal-to-noise resolution and poor reproducibility of this peak precluded analysis. Similar to protonated purple bacterial reaction centers reconstituted with deuterated quinone [48], the high field absorption normally found in fully deuterated PS I is absent with these DK_1-reconstituted samples. Signal intensity increased dramatically from the PK_1- to the DK_1-reconstituted samples. In all cases in which an ESP EPR signal was observed, signal intensity was strongly dependent upon the detection phase (at 500 Hz light modulation). Phase behavior was similar for all spinach PS I samples. The addition of sodium ascorbate assisted in electron cycling (probably functioning by assisting $P_{700}^+A_1^-$ reversibility) and was required for uniform phase behavior, and to observe maximum signal intensity [51].

In Fig. 1 we compare the ESP EPR signals for the DK_1-reconstituted sample, for a LM-EPR scan collected in the derivative mode, with the mathematical derivative of a TR-EPR scan collected at 1 μs after laser excitation. From the shapes of the time-resolved spectra, we can determine the proper detection phase in the light-modulation experiments. We see that overall shapes of the spectra are very similar and, therefore, LM-EPR is a valid technique for investigating ESP EPR in PS I.

The results of reconstitution with a series of quinones and quinone analogues on ESP EPR signals are summarized in Table I. When the PS I samples were reconstituted with NQ or Cl_2NQ, linewidths were reduced significantly (Fig. 2). In addition, *g*-factors in each spectrum were shifted with respect to the other spectrum and with respect to the PK_1-reconstituted spectrum. ESP EPR signals were not restored in PS I samples which were reconstituted with quinones that either were more difficult to reduce than K_1 (e.g., $NC_{14}NQ$) or lacked sufficient hydrophobicity (e.g., BQ, see Fig. 2).

We can immediately deduce that requirements for electron transfer to the quinone from A_0^- are not as

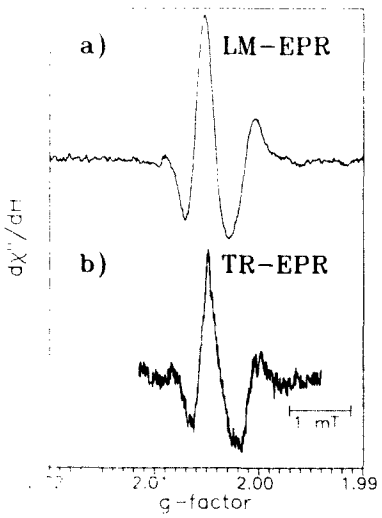

Fig. 1. A comparison of X-Band ESP EPR spectra for a PS I sample reconstituted with 50 μM DK_1 collected by two different methods. (a) LM-EPR signals collected at 500 Hz light modulation, 0.5 mW microwave power, and 0.125 mT modulation amplitude with 12 min of signal averaging. (b) TR-EPR signals were collected by direct-detection in the absorption mode. Samples were excited with a laser operating at 585 nm, 20 Hz, and 5 mJ/pulse. The EPR settings were: 1 mW power, modulation amplitude off, and 8 min of signal averaging. The mathematical derivative of the signal is presented for a 1 μs time delay with a 300 ns window. The shape of the LM-EPR spectrum is very similar to the TR-EPR spectrum. The left side of the spectrum represents the low-field region.

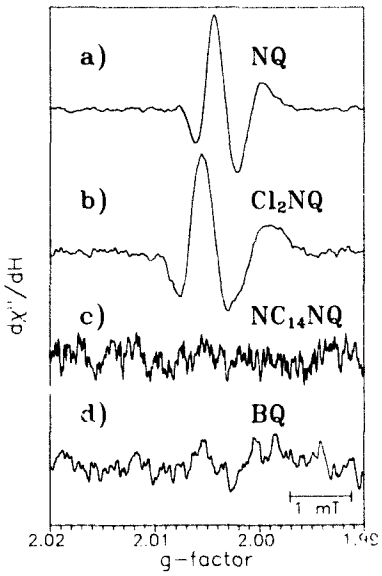

Fig. 2. ESP EPR signals for extracted PS I samples reconstituted with 50 μM quinone (collected under the same conditions as Fig. 1a. (a) NQ; (b) Cl_2NQ; (c) $NC_{14}NQ$; (d) BQ. Both NQ and Cl_2NQ restored the ESP EPR signal with significant line narrowing. The *g*-factors are shifted with respect to each other for these two samples. The ESP EPR signal was not restored by reconstitution with either $NC_{14}NQ$ or BQ.

stringent as electron transfer from the quinone to the FeS centers (P_{430} turnover). P_{430} turnover at room temperature required the presence of an alkyl tail at the 3 position and, possibly, a methyl group at the 2 position on the aromatic ring [22]. Recovery of the ESP EPR signal required an acceptor incorporating either an alkyl tail on a single aromatic ring (i.e., benzoquinone) or at least two aromatic rings (i.e., naphthoquinone). We disagree with recently published work in which the ESP EPR signal was restored with duroquinone [34]. This discrepancy might be attributed to either the large difference in reconstituted quinone concentrations employed (1 mM in comparison to our 50 μM), or the difference in EPR temperature employed (room temperature in comparison to our cryogenic temperature). We estimate that the quinone must have a solution reduction potential within about 50 mV of K_1 (−710 mV) to restore P_{430} turnover, while the ESP EPR signal could be restored by any quinone (that also met the hydrophobicity requirements) with a reduction potential more positive than about −750 mV.

Signals from several samples (e.g., NQ and Cl_2NQ) exhibited significantly narrowed linewidths and increased intensities, in comparison to either the control or PK_1-reconstituted sample (Fig. 2), confirming the results of Sieckman et al. [34]. The line narrowing can be attributed to a reduction in the total number of protons that contribute to the inhomogeneous line-broadening by hyperfine coupling. For instance, for NQ, most of the hyperfine line-broadening arises from interactions with two protons (at the 2 and 3 positions) whereas in PK_1 broadening arises primarily from five protons (three from the methyl ligand and two from the first methylene unit on the phytyl ligand). For radical pairs with correlated radical pair polarization (CRPP), narrow lines will produce relatively intense signals [48,52]. This intensity increase can be attributed to a combination of a smaller range of peak integration and also to decreased signal cancellation of overlapping emission and absorption peaks. Since the ESP EPR signal intensities are dependent upon such factors, we cannot use relative signal intensities to determine relative binding strengths until complete spectral simulations are performed.

Electron transfer from the quinone to the FeS centers is more selective than electron transfer to the quinone. We summarize DMF solution reduction potentials in Table I. This is not surprising in cases in which the reconstituted quinone is much easier to reduce than the endogenous K_1 (e.g., NQ). As a donor, A_0^- has sufficient reducing strength to transfer an electron to the reconstituted quinone while, on the other hand, after reduction the reconstituted quinone cannot reduce the FeS centers (no P_{430} turnover) [22]. In cases in which the reconstituted quinone has a reduction potential similar to K_1, but electron transfer to the FeS centers is not observed (e.g., Me_2NQ), structural factors can potentially limit electron transfer (e.g., the ability of the quinone to function in the electron transport pathway is dependent upon its hydrophobicity) [22]. A long alkyl tail is required in the 2 or 3 positions of NQ for electron transfer to proceed beyond the quinone and ultimately induce P_{430} turnover. Several functions for the alkyl-tail ligand can be envisioned. It may increase quinone binding to the A_1 site, acting as an anchor that holds the quinone in the correct orientation within the binding site for electron transfer to and from the quinone. Another possi-

TABLE II

Exchange of quinones in untreated PS I

Added Quinone	Concentration (μM)	Incubation time (h:min)	37°C [a]	Illumination (10 min) [b]	ESP EPR signal [c]
Control	no add	24:00			control
–	no add	01:10	+		control
–	no add	00:10		+	control
PK_1	100	15:00			control
DK_1	50	00:10			control
–	50	24:00			40%D
–	50	00:10		+	100%D
–	50	00:10	+		>100% D
–	10	00:10		+	40% D
NQ	100	15:00			control
–	100	48:00			control
Cl_2NQ	50	00:10			control
–	50	00:10		+	control

[a] Incubation at 37°C, otherwise at 4°C.

[b] Otherwise incubated in the dark.

[c] Percent of deuterated character in the quinone portion of the ESP EPR signal in a relative comparison to DK_1-reconstituted samples. This represents a qualitative comparison between the signals.

ble role for the alkyl tail is to increase the binding of the reduced semiquinone to the A_1 site. In a hydrophobic environment, the semiquinone would have a diminished binding affinity and, without the hydrophobic interactions of the alkyl tail, could be ejected from the binding site before electron transfer to the FeS centers could occur. Alternatively, decreased semiquinone binding might result in a loss of orientation of this semiquinone donor within the binding pocket, interrupting electron transfer to the FeS centers. The role of donor/acceptor orientation must be addressed by modeling the ESP EPR signals observed in the control and reconstitution experiments. Preliminary modeling indicates that the reconstituted PK_1 lies in about the same orientation as the endogenous quinone in the control sample while the smaller Cl_2NQ and NQ are rotated into different positions in the binding site [53] (in agreement with Sieckman, et al. [34]). Since the isotropic g-factors for the Cl_2NQ ($g = 2.0044$) and NQ ($g = 2.00437$) semiquinones are very similar [53], the shifts observed in Fig. 2 must be attributed to either a change in anisotropic g-factors and/or changes in quinone orientation.

Quinone exchange in PS I

The results of the ESP EPR experiments in which the quinone was exchanged with the endogenous PK_1 (as opposed to reconstituted in quinone-depleted samples) are summarized in Table II and in Figs. 3 and 4. When the control sample was incubated overnight with 100 μM PK_1, the ESP EPR signal was not perturbed (Table II). Therefore, quinones which did not noticeably alter the ESP EPR spectra in the reconstitution experiments (in comparison to the control sample) were not investigated by exchange since there would be no experimental indication of quinone replacement. The control sample was incubated with 50 μM DK_1 in the dark at 4°C. After 10 min of incubation (the time required to dark-adapt the sample), the ESP EPR signal retains the shape and relative intensity of the untreated (PK_1) sample (Fig. 3b). About 2 h of incubation at 4°C were required to noticeably perturb the ESP EPR spectrum. After 24 h of incubation with DK_1 (in the dark at 4°C), the ESP EPR signal exhibits a line-narrowing and intensity increase similar to the signal observed with DK_1-reconstituted samples (Fig. 3c). We qualitatively compared the ESP EPR signal intensities and lineshapes of the DK_1-exchanged sample to the PK_1- and DK_1-reconstituted samples. From this comparison we estimate that the quinone portion of the ESP EPR signal has developed almost half deuterated character and, therefore, almost one half of the PK_1 in A_1 sites (that contribute to the ESP EPR signal) have been replaced by DK_1. Initial ESP EPR investigations with perdeuterated *Synechoccus lividus* PS I particles indicate that we can replace endogenous

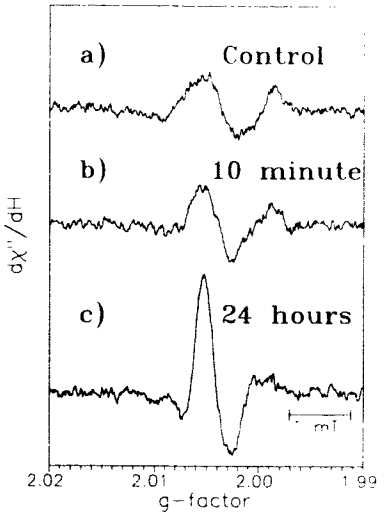

Fig. 3. ESP EPR signals for control PS I samples in which the quinone was 'exchanged' with 50 μM DK_1 (collected under the same conditions as Fig. 1a). Exchange was carried out at 4°C in the dark. (a) 'Untreated' control sample; (b) 10 min exchange; (c) 24 h exchange. After 10 min of exchange, the ESP EPR signal looks identical to the control sample while after 24 h, the quinone portion of the ESP EPR signal has developed some of the characteristics of the DK_1-reconstituted sample (Fig. 1a). Note that relative signal intensities are identical for all three spectra.

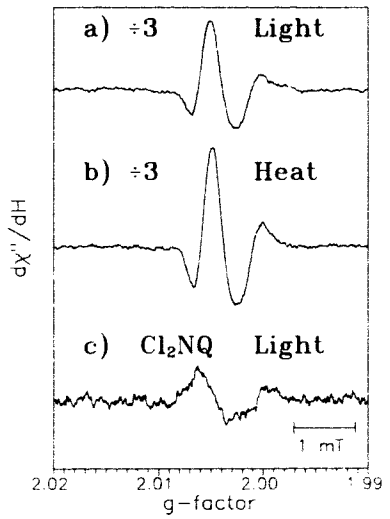

Fig. 4. ESP EPR signals for control PS I samples in which the quinone was 'exchanged' (collected under the same conditions as Fig. 1a). (a) Exchange with 50 μM DK_1, carried out for 10 min at 4°C while illuminating with a projector lamp; (b) Exchange with 50 μM DK_1, carried out for 10 min at 37°C in the dark. After either light or heat treatment, the quinone portion of the ESP EPR signal has completely developed the deuterated characteristics of the DK_1-reconstituted sample (Fig. 1a). (c) Exchange with 50 μM Cl_2NQ, carried out for 10 min at 4°C while illuminating with a projector lamp. For a and b, note that relative signal intensities are three times greater than relative intensities in Fig. 3. Relative signal intensities in (c) are identical to relative intensities in Fig. 3.

DK_1 with exogenous PK_1 by quinone exchange (unpublished results). With PK_1 exchange, signal intensities were severely attenuated and linewidths broadened.

In Fig. 4, we present the results of 10 min DK_1-exchange treatments in which we either illuminated the sample while immersed in an ice bath (Fig. 4a) or incubated the sample in the dark at 37°C (Fig. 4b). Relative signals intensities in Fig. 4a and b are much greater than in Fig. 3, and, therefore, intensity scales have been adjusted by a factor of 3. Both incubation at 37°C and illumination increased DK_1 exchange rates. In comparison with the signals from the reconstituted samples, we estimate that in both the illuminated and the 37°C samples, the quinone portion of the ESP EPR signals appears to be almost completely deuterated and, therefore, that almost all of the endogenous quinone has been replaced by DK_1. In fact, the signal from the 37°C sample was even stronger than from the DK_1-reconstituted sample, but since we are comparing different preparations (control vs. the solvent extracted sample) this result is not quantitatively significant. The ESP EPR signal was not perturbed when the control sample (no quinone added) was either incubated at 37°C for 70 min or illuminated for 10 min (Table II).

Signals were not perturbed when PS I samples were incubated with either Cl_2NQ or NQ (Table II). Neither extended incubation times nor illumination aided exchange of these quinones (Fig. 4c). If either quinone had replaced the endogenous quinone, they would be expected to perturb the ESP EPR signal (see Fig. 2a and b). No changes were observed and we conclude that neither of these two quinones that lack an alkyl tail, were able to replace endogenous K_1.

DK_1 will have about the same binding constant to the A_1 site as PK_1. From partitioning experiments between aqueous and organic solvents [37,38], we assume that all of the K_1 will bind to the photosynthetic membrane. In addition, the deuterated quinone is present at a much higher concentration than endogenous PK_1. In a direct competition between the endogenous quinone and added DK_1, we expect that any unoccupied A_1 sites will more likely be filled by the more concentrated DK_1. Therefore, the DK_1-exchange experiment directly probes quinone release from the A_1 binding pocket and exchange kinetics are determined by quinone exit rates. From both the short duration incubation experiments with DK_1 and the longer duration incubation with NQ, we conclude that quinones situated outside of the A_1 binding site do not participate in electron transfer at rates comparable to electron transfer to species coordinated to the A_1 site. If quinones not bound at A_1 were involved in electron transfer, the large excess of exogenous quinone would dominate the ESP EPR signal. Therefore, the observed ESP EPR signal in the extraction/reconstitution samples probably arises from quinones situated in the A_1 binding pocket, not outside of it. However, these results do not preclude the creation of new binding sites that participate in electron transfer after the hexane/methanol extraction treatment. The increased exchange rate at 37°C is indicative of exchange dependence upon quinone diffusion out of A_1. Enhanced quinone exchange with illumination could be attributed to either a local heating effect, or, possibly, decreased binding of the photoinduced semiquinone.

Quinone exchange is a unique indicator of the lability of the A_1 acceptor in PS I. Endogenous quinone cannot be removed from the PS I particle without harsh solvent treatment. Most models of PS I have considered A_1 to be non-labile, similar to the non-labile quinone (Q_A) in purple bacterial RC's and Photosystem II (PS II) [54,55]. From the exchange results, we conclude that the A_1 quinone of PS I is somewhat labile and has properties similar to the labile quinone (Q_B) in bacterial RC's and PS II [54–57]. The physiological role of K_1 lability in PS I is unclear at this time. From the increased exchange rates observed during illumination, we speculate that quinone lability might play a role in light regulation of PS I during intense light flux conditions.

The results of the quinone exchange experiments suggest that the A_1 binding site in PS I might be a good herbicide target [58,59]. Traditional green plant herbicides act by interrupting electron transfer at the Q_B site in PS II, in conjunction with a pool of plastoquinone molecules [55–57,60]. Since K_1 is present at much lower concentrations than plastoquinone, PS I-based herbicides might function effectively at much lower concentrations. We are currently investigating possible herbicides that will target the A_1 site and interrupt PS I electron transfer.

Conclusions

We demonstrated that the ESP EPR signal in PS I previously attributed to a radical pair composed of P_{700}^+ and A_1^- (where A_1 is the quinone K_1), is restored in K_1-depleted samples by reconstitution with a variety of quinones. Conditions to restore electron transfer to the quinone are much less stringent than to restore electron transfer from the quinone to the FeS centers. Quinone reduction only requires that the added acceptor be more easily reduced than K_1, while electron transfer to the FeS centers requires that the reduction potential of the added quinone be poised near the reduction potential of the native quinone. Binding requirements are also less stringent for quinone reduction than for electron transfer from the quinone to the FeS centers. Quinone reduction requires only sufficient acceptor hydrophobicity while electron transfer to the FeS centers requires both a NQ ring structure and an alkyl-tail ligand in the 2 or 3 positions. Inter-

ruption of electron transfer to the FeS centers, when employing a quinone lacking an alkyl tail, might result from either an orientation loss of the quinone (or semiquinone) from the A_1 site or from ejection of the semiquinone from the binding pocket.

We established that endogenous K_1 occupying the A_1 site in PS I is somewhat labile and can be replaced by exogenous DK_1. This lability is not manifested in terms of depletion of K_1 from the PS I particle but, instead, represents loss from the A_1 site into the photosynthetic membrane. Exchange is not observed with quinones that are expected to bind more weakly than K_1. Exchange rates are enhanced by either heating or illumination. The dependence upon illumination might be an indicator that exchange occurs while the acceptor is in a photoinduced semiquinone form. We speculate that the quinone lability might play a role in PS I light regulation.

Acknowledgments

We thank Walter Svec, Henry Crespi and Tomasz Michalski (Argonne National Laboratory) for growing deuterated algae and extraction of deuterated vitamin K_1. We thank Paul Melnicoff (Argonne National Laboratory) for running the mass spectroscopy, George Gaines (Argonne National Laboratory) for suggesting the light-induced exchange experiments, and Dietmar Stehlik (Free University of Berlin) for use of the EPR simulation program. This work was supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Chemical Science under contract W-31-109-Eng-38, Competitive Research Grants Office of the USDA (88-37130-4135), and the National Science Foundation (DMB-86-03586).

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