Recruitment of a Foreign Quinone into the A₁ Site of Photosystem I

CONSECUTIVE FORWARD ELECTRON TRANSFER FROM A₀ TO A₁ TO Fₓ WITH ANTHRAQUINONE IN THE A₁ SITE AS STUDIED BY TRANSIENT EPR

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In photosystem I (PS I), phylloquinone (PhQ) acts as a low potential electron acceptor during light-induced electron transfer (ET). The origin of the very low midpoint potential of the quinone is investigated by introducing anthraquinone (AQ) into PS I in the presence and absence of the iron-sulfur clusters. Solvent extraction and reincubation is used to obtain PS I particles containing AQ and the iron-sulfur clusters, whereas incubation of the menB rubA double mutant yields PS I with AQ in the PhQ site but no iron-sulfur clusters. Transient electron paramagnetic resonance spectroscopy is used to investigate the orientation of AQ in the binding site and the ET kinetics. The low temperature spectra suggest that the orientation of AQ in all samples is the same as that of PhQ in native PS I. In PS I containing the iron-sulfur clusters, (i) the rate of forward electron transfer from the AQ− to Fₓ is found to be faster than from PhQ− to Fₓ and (ii) the spin polarization patterns provide indirect evidence that the preceding ET step from A₀− to quinone is slower than in the native system. The changes in the kinetics are in accordance with the more negative reduction midpoint potential of AQ. Moreover, a comparison of the spectra in the presence and absence of the iron-sulfur clusters suggests that the midpoint potential of AQ is more negative in the presence of Fₓ. The electron transfer from the AQ− to Fₓ is found to be thermally activated with a lower apparent activation energy than for PhQ in native PS I. The spin polarization patterns show that the triplet character in the initial state of P₇₀₀AQ− increases with temperature. This behavior is rationalized in terms of a model involving a distribution of lifetimes/redox potentials for A₁ and related competition between charge recombination and forward electron transfer from the radical pair P₇₀₀A₁−.

In oxygenic photosynthesis, photosystem I (PS I) and photosystem II (PS II) act in tandem to oxidize water and to reduce NADP⁺ to NADPH on the luminal and stromal sides of the thylakoid membrane, respectively. Both photosystems use light to drive the transfer of an electron from a chlorophyll dimer as donor on the luminal side of the complex via an intermediate acceptor to a quinone on the stromal side. In PS I, the chlorophyll dimer is referred to as P₇₀₀, the intermediate is a chlorophyll monomer, called A₀, and the quinone, A₁ is phylloquinone (PhQ). In PS II, the chlorophyll dimer is P₆₈₀₀, the intermediate is pheophytin, and the quinone, Qₐ is plastoquinone-9. The x-ray structures of the two complexes (1–4) show that the structural arrangement of these co-factors is very similar with two nearly symmetric branches of acceptors extending across the membrane from the chlorophyll dimer. The structures also reveal an accessory chlorophyll monomer located between P₆₈₀₀ and pheophytin in PS II and between P₇₀₀ and A₀ in PS I. The function of these accessory chlorophylls is uncertain at present, but it is likely that they are involved in the initial charge separation acting as either primary acceptor or primary donor. Despite these similarities, the redox potentials of the radical ions generated by the electron transfer are very different in the two systems (see Ref. 5 for a comparison). On the luminal side, the redox couple P₆₈₀₀/P₆₆₀₀ in PS II has a midpoint potential, which is about half a volt more positive than that of P₇₀₀/P₇₀₀ in PS I. On the stromal side, the redox potential of A₁/A₁− in PS I is more negative than that of Qₐ/Qₐ again by at least half a volt. Thus, oxygenic photosynthetic organisms appear to have evolved two versions of essentially the same complex with PS II being optimized to produce the strong oxidizing potential required to split water in the lumen, whereas PS I has been optimized to produce a very negative reduction potential in the stroma. In this respect, phylloquinone, which is a bound intermediate and acts as a strong reductant in the electron transfer chain, is unusual. Quinones more typically function as mobile electron and proton carriers, and in the thylakoid membrane they act primarily as a sink (quinone pool) for the electrons transferred through PS II (6). Of particular interest is the origin of the large difference in the single reduction midpoint potentials of phylloquinone as A₁ in PS I and plastoquinone as Qₐ in PS II. Experiments in which

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The abbreviations used are: PS I, photosystem I; PS II, photosystem II; bRC, bacterial reaction center(s); PhQ, phylloquinone; A₀, 9,10-anthraquinone; Qₓ, quinone in the A₁ site of PS I; FeS, four iron-four sulfur cluster, either FX, Fₓ, or Fₓ, P₇₀₀ chlorophyll a/a’ heterodimer that represents the primary electron donor; A₀ (chlorophyll a) and A₁ (quinone), sequential electron acceptor sites in PS I; TR EPR, time-resolved or transient electron paramagnetic resonance; A, absorptive EPR signal; E, emissive EPR signal; hfc, hyperfine coupling; ET, electron transfer; Tricine, N-[2-hydroxy-1,1-bis(hydroxymethyl)-ethyl]glycine.
the same quinone is incorporated into both photosystems show that the difference is induced almost completely by the respective protein environment. This is demonstrated most clearly by the fact that when plastoquinone is incorporated into the A₁ site in PS I, its reduction midpoint potential is several hundred mV more negative than when it is in the QA binding site in PS II (7). By comparison, the difference in midpoint potentials of PhQ and PQ (less than 100 mV) is much smaller. Although the redox potential of the quinone in a given reaction center is largely determined by the respective protein environments, the molecular structure of the quinone itself is of some importance. Introducing a series of structurally related quinones allows the redox potential of the electron transfer co-factor to be varied systematically so that its influence on the kinetics can be studied. Extensive investigations of this type have been carried out on purple bacterial reaction centers (bRCs) (8–13); however, there have been relatively few studies on PS I. Itoh and co-workers (14) studied the kinetics of electron transfer from the chlorophyll acceptor A₀ to a series of acceptors in the A₁ site. They estimated ΔΓ for the electron transfer (ET) from A₀ to A₁ in each case, and using the ET rates measured by absorbance difference spectroscopy, they constructed a Marcus plot for the reaction. From the plot, it was concluded that the native PhQ gives the optimal ET rate (14, 15). The forward electron transfer from A₁ to F₅ in PS I containing nonnative quinones has also been investigated (7, 16–21). However, a reliable relationship between ΔΓ and the rate of this reaction has not been established. In nearly all cases studied so far, the midpoint potential of the nonnative quinones has been more positive than PhQ, and the rate of forward electron transfer from A₁ to F₅ was slower than in native PS I or even blocked. In contrast, the effect of pushing the quinone midpoint potential to values more negative than in the native system has not been investigated yet.

In this work, we use transient EPR to investigate the effect on the electron transfer kinetics of introducing anthraquinone (AQ) into the A₁ site. This will allow us to study the correlated effect on the rate of electron transfer from A₀ to A₁, and from A₁ to F₅, when the midpoint potential of the acceptor in the A₁ is more negative than that of PhQ. We will show that the electron transfer rate from AQ to F₅ is faster than in native PS I, and to the best of our knowledge this is the only example of a nonnative acceptor that demonstrates faster electron transfer than the native one in a photosynthetic reaction center. On the other hand, the more negative redox potential of AQ is also expected to slow the preceding electron transfer step from A₀ to A₁. If the lifetime of the primary radical pair becomes long enough, singlet-triplet mixing will occur and will influence the spin polarization of all subsequent radical pairs (22–26). Such effects have been studied extensively using transient EPR on bRCs containing nonnative quinones in the QA site (23, 24, 27–32) but have not yet been observed in the spin polarization patterns of PS I with nonnative acceptors in the A₁ site. Here, we will demonstrate that with AQ in the A₁ site, the transient EPR spectra show clear evidence for singlet-triplet mixing in the primary radical pair.

**MATERIALS AND METHODS**

**Quinones—**Quinones and the reagents for the preparation of buffers were purchased from Aldrich. Commercial anthraquinone was purified by column chromatography on aluminum.

**Preparation of Photosystem I Samples—**PS I isolated from *Synechocystis* PCC 6803 was prepared by the method of Biggins and Mathis (16). The isolated PS I was lyophilized, and the native phylloquinone was removed by solvent extraction using hexane (99%; Aldrich) containing 0.3% methanol (Caledon) as also described in Ref. 17. The extraction of the phylloquinone was monitored using a modified Bruker ESP 200 X-band spectrometer (described below) by measuring the disappearance of the spin polarized EPR signal due to P₇₀₀⁻AQ⁺ and the accompanying appearance of the characteristic polarization pattern of PhQ⁺ formed by recombination from P₇₀₀⁻AQ⁺. The loss of other pigments was monitored using the visible absorption spectrum of the supernatant recorded with a Unicam UV-visible spectrometer. Samples for EPR analysis were prepared by suspending 15 mg of extracted PS I in 150 μl of buffer containing 50 mM Tricine, 10% glycerol, and 0.2% Triton X-100. A homogenizer was used to ensure complete resuspension. Anthraquinone was introduced into the A₁ binding site by adding ~5 μl of a ~150 mM solution of AQ in Me₂SO to the resuspended PS I sample and in the dark at 4 °C for several hours. The incorporation of quinone into the A₁ site was monitored by observing the appearance of a spin polarized EPR spectrum due to P₇₀₀⁻AQ⁺ accompanied by a decrease in the intensity of the P₇₀₀⁺ spectrum. A control experiment using a blank of the solvent containing no quinone was also performed, and no change in the transient EPR spectra was observed. For the EPR experiments, 1 mM sodium ascorbate and 50 μM phenazine methosulfate were added as external redox agents, and the samples were frozen in the dark.

**Transient EPR Spectroscopy—**Control experiments and monitoring of the extraction and reconstitution of the quinone were carried out at X-band using a modified Bruker ESP 200 spectrometer equipped with a home-built, broad band amplifier (bandwidth >500 MHz). A rectangular resonator and a liquid nitrogen temperature control unit were used, and the samples were illuminated using a q-switched, frequency-doubled Continuum SureLite Nd-YAG laser at 532 nm with a repetition rate of 10 Hz.

All other X-band transient EPR experiments were carried out using a Bruker ER046 XK-T microwave bridge equipped with a Flexline dielectric resonator (34) and an Oxford liquid helium gas flow cryostat. The loaded value for this dielectric ring resonator was about Q = 3000, corresponding to a rise time of τ_r = Q/(2π × v_m) ~ 50 ns. Q-band (35-GHz) transient EPR spectra of the samples were also measured with the same set-up except that a Bruker ER 056 QMV microwave bridge equipped with a home-built cylindrical resonator with access for light irradiation was used. The samples were illuminated using a Spectra Physics Nd-YAG/MOPO laser system operating at 10 Hz at either the second harmonic 532 nm or near the long wavelength absorption edge of PS I at ~700 nm.

**RESULTS**

**Efficiency of Incorporation of AQ into the A₁ Binding Site—**When native PhQ is extracted from PS I, the spin polarization pattern of the P₇₀₀⁺ state, formed by recombination of the primary radical pair state P₇₀₀⁻A₁⁰⁻, is observed. The relative amplitudes of the spectra of P₇₀₀⁺ versus P₇₀₀⁻A₁⁰⁻ provide a convenient measure of the remaining occupancy of the A₁ site, which was used to monitor the quinine extraction (see Fig. 1 of Ref. 35). The incorporation of AQ into the A₁ site during quinone reconstitution can also be monitored in this way. However, as we will discuss below, the spin polarization patterns suggest that the electron transfer from A₀ to AQ is inefficient and that some charge recombination to P₇₀₀⁺ probably occurs even when the A₁ site is occupied. Thus, only the functional occupancy of the A₁ site can be estimated from the amplitude ratio of the P₇₀₀⁺ and P₇₀₀⁻AQ⁺ spectra. As shown in the accompanying paper (36), AQ has a lower affinity for the A₁ site compared with other quinones. Consistent with this, the P₇₀₀⁺ spectrum is not lost completely when extracted PS I is incubated with AQ. Nonetheless, the transient EPR spectra of P₇₀₀⁻AQ⁻ can be recorded with a good signal/noise ratio for detailed analysis.

**Low Temperature Transient EPR Spectra of P₇₀₀⁻AQ⁻**—Fig. 1 shows a comparison of X-band (top) and Q-band (bottom) spectra of P₇₀₀⁻PhQ⁻ in native PS I and P₇₀₀⁻AQ⁻ in solvent-extracted PS I reconstituted with AQ. Simulations of the spectra (dashed curves) are also shown. As can be seen in Fig. 1, the transient EPR spectra of both samples exhibit the same overall polarization pattern (i.e. E/A/E (where E represents emission and A is absorption) at X-band and E/A/E/A/E at Q-band). Note that the total spectral width is lower for P₇₀₀⁻AQ⁻. This is consistent with the smaller g-tensor anisotropy of AQ and the absence of the partially resolved methyl hyperfine coupling.
A quinone (AQ) in the A1 site of Photosystem I (PS I) at 80 K.

The methyl hyperfine coupling (hfc) is strongest along the C–CH₃ bond, which contributes to the spectral width of the X-band spectrum of P700$^{+}$ (hfc), which represents a major contribution to the spectral width of the X-band spectrum of P700$^{+}$, native PS I (37–40). The methyl hfc is strongest along the C–CH₃ bond, which is close to the principal γ axis of the PhQ$^{−}$ g-tensor. When the magnetic field is along this axis, the methyl hfc is partially resolved as seen on the central absorptive feature of the P700$^{+}$PhQ$^{−}$ spectrum. The methyl hfc is most noticeable as a shoulder on the central absorption at X-band and as a series of small peaks in the Q-band spectrum. AQ has no methyl substituent, and the hfc of the outer ring protons are expected to be small. Correspondingly, the P700$^{+}$AQ$^{−}$ spectrum shows a much narrower absorptive feature in the center of the spectrum.

The hfc's for quinones in the A1 site in PS I are different from those observed in polar solution or reaction centers of purple bacteria. These differences provide a sensitive indicator for the asymmetry of the spin density distribution in the different environments, and from this observed asymmetry the asymmetry of hydrogen bonding can be deduced (20, 41, 42). A dominant hydrogen bond to the carbonyl group ortho to the phytly tail and meta to the methyl group leads to an increased hfc constant for the methyl protons (35, 39, 40). In the A1 site, AQ is expected to bind with the same highly asymmetric bonding scheme as PhQ. As a consequence, high spin density is predicted at the outer ring carbon position, which is two carbon atoms away from the hydrogen-bonded carbonyl group. The protons at these two positions have their bond axes along the molecular x direction. The dominant hyperfine axis of an aromatic C–H fragment is expected to be in-plane and perpendicular to the C–H bond (43); thus, the largest coupling would be expected along the molecular y axis. However, the hyperfine couplings for AQ are small and cannot be resolved directly in the EPR spectrum. Pulsed ENDOR spectroscopy will be used to confirm the same asymmetric hydrogen bonding scheme for AQ in the A1 site as established for native PhQ.

Quinone-protein interactions are also reflected in differences of the observed quinone g-tensor components in different environments (e.g., solution, native A1 site, A1 sites modified by mutation) (44–47). Unfortunately, both hydrogen bond formation and π stacking lead to quite small and generally opposite (i.e., compensating) shifts in the g-tensor anisotropy (38). Comparison of the AQ g-tensor in PS I versus bRC (31) (Table I) shows a larger g-tensor anisotropy in PS I. The same has been found for other quinones in PS I versus bRC (45, 49). The analogous qualitative changes in all of these cases provide additional evidence that AQ in PS I experiences very similar protein cofactor interactions (hydrogen bond formation and π stacking) as phyloquinone in the wild type.

The spin polarization pattern of the P700$^{+}$Q$^{−}$ state is known to be particularly sensitive to the relative orientations of the g-tensors g(P700$^{+}$) and g(Q$^{−}$) with respect to the symmetry axis of the dipolar coupling tensor in the radical ion pair (i.e., the vector $z_D$ connecting the spin density centers of the respective radical ions) (50, 51). As shown in Ref. 51, the relative orientations can be determined from the high field EPR radical pair spectra if one of the angles involved can be fixed. Here, we have taken a more conservative approach and simply calculate the X- and Q-band spectra of P700$^{+}$AQ$^{−}$ and P700$^{+}$PhQ$^{−}$ with a set of parameters based on established literature values. For native PS I, we have used the geometric and magnetic parameters given in Ref. 51 and have calculated the spectra according to the algorithm described in detail in Ref. 53. For the spectra of P700$^{+}$AQ$^{−}$, we have assumed the same geometry and adjusted the AQ g-values to those given in Table I. Inspection of the polarization pattern shows a narrower line width for features associated with the molecular y-direction; thus, we assume an anisotropic inhomogeneous line width, which is smaller along the y-direction compared with the x- and z-directions, as indicated in Table I. The good agreement between the simulated and experimental spectra using the same set of geometric parameters in each case suggests that AQ binds in the same orientation as the native PhQ, with the carbonyl bond axes parallel to the vector joining P700$^{+}$ and AQ$^{−}$ ($z_D \parallel x_D$).

**Manifestation of Electron Transfer Kinetics in the Transient EPR Data Sets**—For native PS I, two consecutive spin polarization patterns due to P700$^{+}$PhQ$^{−}$ and P700$^{+}$FeS$^{−}$ are ob-

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**FIG. 1.** X-band (top) and Q-band (bottom) spin-polarized transient EPR spectra of WT PS I (Synechocystis PCC 6803) and A1-extracted PS I with AQ in the A1 site at 80 K. Positive signals correspond to absorption (A), and negative signals correspond to emission (E). The spectra are due to the P700$^{+}$A1$^{−}$ state and have been extracted from the full time/field data sets by numerical integration of the signal intensity in a time window from 152 to 1520 ns following the laser flash. The dashed line spectra are the result of simulations as described under “Results” and with the parameters listed in the Table I. The magnetic field scan range is different for the X-band and Q-band spectra; correspondingly, the overall spectral width at Q-band is larger, as expected, when it is dominated by the respective g-anisotropy.
served at temperatures above \(-240\) K (see Ref. 54 for a review). Although it is known that electron transfer past \(A_1\) proceeds via \(F_X\) (34, 55, 56), the kinetics of electron transfer past \(F_X\) are not well established. Therefore, we use the notation \((FeS)_{AQ}\) to leave open the question of which of the three iron-sulfur clusters is reduced during the chosen time window for TR EPR observation. With nonnative naphthoquinone and benzoquinone derivatives in the \(A_1\) site, forward electron transfer past the quinone is generally slower than the relaxation of the spin polarization, and therefore only \(P_{700}^+Q^-\) is observed (7, 17). In contrast, with \(AQ\) in the \(A_1\) site, two sequential spectra are observed in the temperature range from 160 to 295 K, and the lifetime of the early spectra is close to or below the spectrometer rise time above \(-240\) K. This is demonstrated in Fig. 2, which shows spin-polarized transient EPR spectra and transients for \(PhQ\) and \(AQ\) in PS I at 230 K. The spectra (top) are taken at early (solid curves) and late times (dashed curves). The transients (bottom) are for selected field positions indicated by the arrows below the spectra (top). With \(PhQ\) in the \(A_1\) site, the \((E/A/E)\) polarization pattern of \(P_{700}^+PhQ^-\) dominates the spectra at early times. At late times, an emissive spectrum of \(P_{700}^+\) in the state \(P_{700}^+\) is observed. With \(AQ\) in the \(A_1\) site, two sequential spectra are also observed, but the respective polarization patterns are drastically different. At early times, an \((E/A/A)\) pattern is found with a spectral width consistent with that of \(P_{700}^+AQ^-\), whereas an \((E/A)\) pattern in the \(P_{700}^+\) region is observed at late times. We will describe later how these drastic changes in the polarization patterns can be interpreted as the result of different spin dynamics in the precursor radical pair state \(P_{700}^+AQ^-\). First, however, we will consider the rate of electron transfer from \(AQ^-\) to \(F_X\).

At the position marked by \(arrow a\) in Fig. 2 (top), there is no significant contribution from \(P_{700}^+\), and the transient signal is due only to \(Q^-\). The lifetimes associated with the decay curves for this position shown in Fig. 2 (bottom) are primarily due to forward electron transfer from \(Q^-\) to the \(FeS\) clusters, with some (minor) contribution due to spin relaxation. The ET kinetics are also reflected in the transients at other field positions (arrows \(b\) and \(c\)), but the behavior is more complicated because contributions from both the \(P_{700}^+Q^-\) and \(P_{700}^+\) overlap. From the comparison of the transients, it is immediately apparent that the decay of the quinone contribution is much faster in the \(AQ\) sample. A fit of the transients shown in Fig. 2 (bottom) using the procedure described in Ref. 34 yields an electron transfer lifetime of 125 ns for the \(AQ\) sample, compared with 840 ns for the \(PhQ\) sample. Thus, we find that with \(AQ\) in the \(A_1\) binding site the electron transfer to the iron sulfur clusters is faster than in native PS I. Since the electron transfer from \(PhQ\) to \(F_X\) is known to be strongly temperature-dependent, we expect the increased rate found for \(AQ\) to be accompanied by a difference in the temperature dependence as well.

**Temperature Dependence of the ET Rate from \(Q\) to \(F_X\)—For PS I reconstituted with \(AQ\), the temperature dependence of the ET kinetics could be clearly resolved between 160 and 240 K. At higher temperatures, it is convoluted with the spectrometer response function; nonetheless, the early spectrum is evident in the data up to 298 K. Below 160 K, the electron transfer rate becomes too slow compared with the spin relaxation rate to be evaluated reliably. As described in Ref. 34, the spectra of \(P_{700}^+Q^-\) and \(P_{700}^+\) can be obtained by fitting a kinetic model to the data and plotting the amplitudes associated with the kinetic components. The spectra obtained in this way are shown in Fig. 3 for the whole set of temperatures between 160 and 240 K. The electron transfer lifetimes obtained from the fit are listed on the left of Fig. 3 beside the corresponding spectra. They are also plotted in Fig. 4 (filled dots) as the natural logarithm of the rate versus the inverse temperature (Arrhenius plot). The lifetimes measured at temperatures above 240 K are given in the legend to Fig. 4. The upper ends of the error bars on these data points represent the spectrometer detection limit, and the lower ends correspond to twice the rise time. For comparison, Fig. 4 also includes corresponding data for native \(PhQ\) (open circles). The solid line represents the temperature dependence observed in transient absorption difference data for native PS I particles as reported in the literature (57, 58). As can be seen in Fig. 4, the EPR and optical data for the slow phase of native PS I agree well (open circles, solid line). A fast kinetic component with a very low activation energy is also seen optically but is not observed by EPR. In cyanobacterial PS I, it accounts for at most a minor fraction of the electron transfer (47, 60, 61). Fig. 4 reveals two main

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**Table 1**

| \(g\)-Tensor | \(g_{xx}\) | \(g_{yy}\) | \(g_{zz}\) | References/sources |
|--------------|-----------|-----------|-----------|-------------------|
| \(P_{700}^+\) | 2.0031    | 2.0026    | 2.0022    | Refs. 40 and 71–73 |
| \(A_1^-\)   | 2.0031    | 2.0031    | 2.0031    | Refs. 75–77       |
| \(PhQ^+\)   | 2.0063    | 2.0051    | 2.0022    | Refs. 37, 40, and 78 |
| \(AQ^+\)    | 2.0059    | 2.0048    | 2.0022    | This work         |
| \(2-ethyl-AQ^+\) | 2.0056  | 2.0049    | 2.0022    | In hRC (31, 38)   |
| \(F_X\)     | 1.75      | 1.56      | 2.06      | Ref. 79           |

| Euler angles between \(g(A_1)\) and \(g(FeS)\) | References/sources |
|-----------------------------------------------|-------------------|
| \(\alpha\) | \(\beta\) | \(\gamma\) | |
| milliteslas | 0.32 | 0.32 | 0.46 | 30° | 0° | 0° | Refs. 33, 59, and 74 |

**References/sources a** Gaussian half-width. For \(AQ\), the line width is assumed to be anisotropic with the given values for the three principle directions of the \(g\)-tensor, respectively.
features of the electron transfer from the quinone to \( F_{X} \) in the samples containing AQ. (i) The electron transfer rate is faster than the dominant component in native PS I by close to an order of magnitude. (ii) Using transient EPR, the ET can be followed down to much lower temperatures than for native PS I because the rate is faster and less dependent on temperature.

**Evidence for Altered Forward ET Past \( A_{0} \)**—In a series of sequential radical pairs, the polarization pattern of any given radical pair is sensitive to the singlet-triplet mixing in all preceding pairs. Since the spin dynamics depend on the lifetimes of the individual radical pairs, changes in the ET kinetics can be reflected in the spectra. Thus, in general, the spectra of \( P_{700}^{+}A_{1}^{-} \) and \( P_{700}^{+} \text{(FeS)}^{-} \) can be expected to change with temperature if the lifetime of a radical pair state happens to pass

![Fig. 2](image-url)  
**Fig. 2.** X-band spin-polarized transient EPR signals taken at 230 K from organic solvent extracted PS I following reconstitution with either PhQ or AQ. **Top frame**, digital boxcar spectra at two different delay times after the laser flash. The early spectra (solid curves) are dominated by \( P_{700}^{+}Q^{-} \), whereas the late spectra (dashed curves) are mainly due to \( P_{700}^{+} \text{(FeS)}^{-} \). Time windows were as follows: PhQ early, 625–750 ns; PhQ late, 2.50–2.75 \( \mu \)s; AQ early, 190–310 ns; AQ late, 0.94–1.2 \( \mu \)s. **Bottom frame**, selected transient EPR kinetic traces taken at the field positions indicated by the arrows labeled a, b, and c below the spectra in the top frame. The faster ET rate with AQ in the \( A_{1} \) site (solid line) is readily recognized in transient (b) by the faster initial turnover from absorption to emission and the minimum at earlier times.

![Fig. 3](image-url)  
**Fig. 3.** Kinetically separated TR EPR spectra of \( A_{1} \)-extracted PS I with AQ reconstituted in the \( A_{1} \) site, at different temperatures in 10-K steps between 160 K (top) and 240 K (bottom). Solid line spectra correspond to the \( P_{700}AQ^{-} \) radical pair state (early signal), and dashed line spectra correspond to the \( P_{700}^{+} \text{(FeS)}^{-} \) state (late signal). The evaluated forward ET lifetimes are given at the left side of the spectra. The value of 100 ns at 240 K compares with \( \sim 1260 \) ns for wild type at this temperature. All of the lifetimes have an error of \( \pm 50 \) ns.

through the time range relevant for singlet-triplet mixing. In fact, in native PS I, such changes are not observed because the primary radical pair state \( P_{700}^{+}A_{0}^{-} \) is too short lived for any significant triplet character to develop during its lifetime, whereas \( P_{700}^{+}A_{1}^{-} \) is sufficiently long lived that the maximum degree of mixing occurs within its lifetime. For PS I containing AQ, the spin polarization pattern of \( P_{700}^{+}AQ^{-} \) is observed to be temperature-dependent, as shown in Fig. 3. Moreover, the spectrum of \( P_{700}^{+} \text{(FeS)}^{-} \) is different from that of native PS I (see Fig. 2, top panel). Both of these observations suggest that singlet-triplet mixing during the lifetime of \( P_{700}^{+}A_{1}^{-} \) state affects the polarization patterns. For such an effect to be observable, the lifetime of \( A_{0}^{-} \) must be longer than \( \sim 0.5 \) ns (53), which corresponds roughly to the inverse of the magnetic interaction parameter (in s\(^{-1}\)) responsible for singlet-triplet mixing. Such a lengthening of the \( A_{0}^{-} \) lifetime is expected for samples in which the redox potential of the quinone is different from that of native PhQ for which ET from \( A_{0}^{-} \) to \( A_{1} \) is optimized as suggested by Iwaki et al. (14). The effect of singlet-triplet mixing in the primary radical pair has been studied extensively by several authors (26, 29, 31, 32, 43, 52, 53, 61, 62), and it is known to result in two additional contributions to the polarization of \( P_{700}^{+} \) in subsequent radical pairs (53). Both of these terms contribute absorptive polarization to the high field end of the \( P_{700}^{+}Q^{-} \) spectrum. As can be seen in Fig. 2, the most striking difference in the polarization patterns of the AQ samples and those of native PS I is the increased absorptive polarization at the high field end of the spectrum and a corresponding decrease of the next absorptive peak toward the center of the pattern. Both features have been shown to arise from an increased lifetime of \( P_{700}^{+}A_{0}^{-} \) (see Ref. 62). To investigate this possibility more carefully, we have simulated the entire time/field data sets for two temperatures and introduced the triplet character of \( P_{700}^{+} \text{(FeS)}^{-} \) as an adjustable parameter expressed as an effective lifetime. The parameters used for the simulations are given in Table I, and the procedure is discussed in Refs. 61 and 63. The results of these simulations are shown in Fig. 5 for
two temperatures. Good agreement is obtained, and the absorptive polarization at the high field end of the spectrum is reproduced correctly by increasing the triplet character of $P_{700}^+AQ^-$. This supports the idea that forward electron transfer from $A_0$ to the quinone is impaired with AQ in the $A_1$ site. However, the changes in the spectra are not easily interpreted in terms of the kinetics because there are a number of possible origins for the triplet character, which are not distinguishable.

In addition to mixing from precession of the spins during the lifetime of the $P_{700}^+AQ^-$, charge recombination from the triplet and singlet state of all of the radical pairs can influence their spin polarization patterns. The fact that the triplet character of the precursor to $P_{700}^+AQ^-$ increases with temperature while the yield of the $^3P_{700}$ signal appears to decrease suggests that competition between different recombination channels may play a role. However, the EPR data do not allow an accurate determination of the relative quantum yields of forward electron transfer and triplet recombination, and independent optical data (64) suggest a very low triplet yield (less than a few percent) in the high temperature region ($>240$ K).

One possible scenario that would explain the temperature dependence of the $P_{700}^+AQ^-$ polarization patterns is a broad distribution of lifetimes for $P_{700}^+AQ^-$ as suggested recently to rationalize similar observations for a point mutant in which the PsaA $A_0$ binding site was altered (61). There is evidence for such a distribution in unmodified reaction centers from purple bacteria (e.g. see Ref. 65), and an increase in the width of the distribution is a likely consequence of mutations in the $A_0$ binding site. In Ref. 61, it was argued that if such a distribution is assumed, the observed spectral patterns can be approximated as the weighted sum of two limiting spectra $S(B(0)_\text{slow})$ and $S(B(0)_\text{fast})$ with a temperature-dependent weighting factor $c(T)$.

$$S(B(0)_T) = c(T) S(B(0)_\text{slow}) + (1 - c(T)) S(B(0)_\text{fast})$$  \hspace{1cm} (Eq. 1)

The limiting spectra represent the cases of either minimum or maximum singlet-triplet mixing in $P_{700}^+AQ^-$ due to fast or slow forward electron transfer from $A_0$, respectively. The increased apparent triplet character in the precursor is then described quite naturally as being due to forward electron transfer outcompeting recombination in a larger fraction of the reaction centers at higher temperature. However, because $S(B(0)_\text{slow})$ can be written as a sum of several terms (53), this model is formally

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**Fig. 4.** Arrhenius plot (ln $k$ versus $1/T$) of the electron transfer rate from AQ to the iron-sulfur cluster in $A_0$-extracted PS I with reconstituted AQ in the $A_1$ site. The lifetimes at $>240$ K are listed beside the corresponding spectra in Fig. 3. The lifetimes above 240 K are as follows: 250 K, 50 ns; 260 K, 50 ns; 298 K, 30 ns. For these three values, the lower limit of the error bars is the detection limit of 20 ns, and the upper limit is taken as 100 ns, which is twice the rise time of the spectrometer. For comparison, the slope of the Arrhenius plot for the PhQ to $F_0$ step in native PS I is included as a straight line. The slope is based on the activation energy: $E_a = 220 \pm 20$ meV evaluated from optical data for Thermosynechococcus elongatus (57) In native PS I, a minor phase with a lifetime of ~10 ns and near zero activation energy has also been reported (58). The two open circles represent the ET rates evaluated from TR EPR data for native PS I (60) at selected temperatures (295 and 260 K) and are in good agreement with the optical data (57). With AQ in the $A_1$ site, the activation energy is clearly lower ($60 \pm 20$ meV below 240 K) than with PhQ. Above 240 K, the electron transfer is faster than the rise time of the spectrometer, and the activation energy is less well defined. However, a least squares fit of the data at 240 K and above gives an activation energy of ~120 meV, suggesting that it may be higher than below 240 K.

**Fig. 5.** Simulations of the polarization patterns of organic solvent-extracted PS I with AQ reconstituted in the $A_0$ site, at two selected temperatures. The simulations are based on the sequential correlated radical pair model (26, 52, 53), and the complete time field data sets have been calculated as described in Refs. 63 and 61. The calculated and experimental spectra are shown at three representative time points in the data set. The magnetic interaction parameters used are given in Table I. In the simulations, a single kinetic phase is assumed, and the triplet character of the primary radical pair has been varied at each temperature by adjusting the effective lifetime parameter, $\tau_{eff}$. At 200 K, $\tau_{eff} = (1.8 \text{ ns}^{-1})$; at 260 K, $\tau_{eff} = (3.4 \text{ ns}^{-1})$. The limiting spectra represent the cases of either minimum or maximum singlet-triplet mixing in $P_{700}^+AQ^-$ due to fast or slow forward electron transfer from $A_0$, respectively.
FIG. 6. Comparison of the X-band spin-polarized TR EPR spectra of the \( P_{700} \) \( AQ^- \) state in two PS I preparations, each for a set of four temperatures between 180 and 240 K. Top, PS I from the \( menB \) \( rubA \) double mutant after incubation with \( AQ \). The time window was 500–750 ns. Bottom, organic solvent-extracted PS I from the wild type reconstituted with \( AQ \). The time window was 250–500 ns. The dashed lines indicate the baseline level to provide a reference for the sign of the spin polarization (positive for \( A \) and negative for \( E \)). Note that the net polarization is at most very small in all spectra.

The Role of \( F_X \) in Determining the Redox Potential of the Quinone—As shown in the accompanying paper (36), \( AQ \) incorporation is also possible with PS I from the \( menB \) \( rubA \) double deletion mutant. The \( rubA \) deletion leads to assembly of PS I particles lacking all three FeS centers (66, 67); hence, it provides an elegant way of investigating the role of \( F_X \) in determining the quinone redox potential. Fig. 6 shows a comparison of spectra of \( P_{700}AQ^- \) in PS I from the \( menB \) \( rubA \) double mutant (top) and in solvent-extracted PS I (bottom). Comparison of the spectra from the two samples shows that the effects attributed to singlet-triplet mixing in the precursor are much less pronounced in the \( menB \) \( rubA \) double mutant (top). Indeed, the spectrum from the \( menB \) \( rubA \) double mutant at 240 K (top) is comparable with the low temperature pattern at 180 K (bottom). This result implies that the slowing of the forward electron transfer through \( A_0 \) is more pronounced when the iron-sulfur clusters are absent. It is important to point out that control experiments in which extracted PS I is reincubated with native PhQ show that the quinone extraction procedure has no influence on the kinetics. The differences between the two samples shown in Fig. 6 would be expected if the midpoint reduction potential of the quinone was more negative in the presence of the iron-sulfur clusters. This is consistent with the fact that the 4Fe4S(SCH$_3$)$_4$ moiety has a formal charge of 2$^\cdot$. Although this charge is probably compensated to a large extent in the protein framework, it should contribute at least a partial negative charge to the local electrostatic environment of the quinone. Hence, the effective redox potential of \( AQ \) in the \( A_1 \) site is expected to be more positive (i.e., closer to that of phylloquinone) when the (FeS) centers are absent. The proposed interpretation in terms of an electrostatic contribution from the iron sulfur centers can be tested experimentally by reconstituting \( F_X \) in the \( menB \) \( rubA \) double mutant as described in Ref. 68. The \( P_{700}AQ^- \) spectra from reconstituted samples (not shown) resemble more closely those from the solvent-extracted PS I, providing support for the notion that the spectral changes are indeed due to the presence or absence of \( F_X \). Further systematic studies using substituted naphthoquinones and anthraquinones are in preparation to confirm these results.

**DISCUSSION**

The results presented above show that when the native PhQ in PS I is replaced with \( AQ \), the following effects are observed: (i) the rate of electron transfer to the iron sulfur clusters is increased by about an order of magnitude at 260 K; (ii) the temperature dependence of the rate is weaker than in native PS I and as a result the electron transfer can be followed down to lower temperature; (iii) the polarization patterns show that the primary radical pair develops significant triplet character; and (iv) the triplet character of the primary radical pair increases with temperature. Notwithstanding the difficulty distinguishing between different origins of the triplet character of the primary radical pair, it is clear that electron transfer from \( A_0 \) to \( AQ \) is slower than from \( A_0 \) to PhQ in native PS I. Thus, for the first time, the effect of the quinone properties on the two consecutive ET steps from \( A_0 \) to \( Qt \) to \( F_X \) can be investigated within the same TR EPR study on the same PS I sample. All of the results are consistent with the idea that a shift of the quinone reduction midpoint potential to a more negative value results in a slowing of the first ET step (\( A_0 \) to \( Q \)), whereas the second step (\( Q \) to \( F_X \)) is accelerated. Marcus theory expresses the rate of a nonadiabatic electron transfer reaction as follows,

\[
k = \frac{2\pi|V|^2}{\hbar} \sqrt{\frac{4\pi\lambda k_BT}{3}} \exp \left[ -\frac{(\Delta G^0 + \lambda)^2}{4\lambda k_BT} \right] \tag{Eq. 2}
\]

where \( V \) represents the electronic coupling between the initial and final states. In general, it is assumed that \( V \) decreases exponentially \((|V|^2 \propto e^{-2R})\) with the edge-to-edge distance \( R \) between the reactants, weighted by an empirical \( \beta \)-factor. \( \lambda \) is the reorganization energy, and \( \Delta G^0 \) is the standard Gibbs free energy of the ET reaction. A plot of log \( k \) versus \( \Delta G^0 \) gives an inverted parabola with its maximum at \( -\Delta G = \lambda \). In PS I, the most extensive set of data from which such a Marcus plot can be constructed is for the ET from \( A_0 \) to \( A_1 \). Itoh and co-workers incorporated a series of acceptors into quinone extracted PS I particles and studied the kinetics using absorption difference spectroscopy (see Ref. 15 for a review). The free energy differ-
ence, $\Delta G^0$, was estimated for each acceptor by assuming a fixed $A_0$ energy level and calculating the midpoint potentials of the acceptors in the A1 site based on their midpoint potentials in DMF (14). The acceptors were chosen so that the $\Delta G^0$ values would cover a range of about 700 mV above and 500 mV below the value estimated for native PS I. Consistent with Equation 2, the experimental plot of $\log k$ versus $\Delta G^0$ gave an inverted bell-like shape, and the optimal barrier-free rate of $\sim 25 \text{ ps}^{-1}$ was found for native PhQ in the A3 site. The redox potential of AQ is estimated to be about 100 meV more negative than that of PhQ. Based on the data given in Ref. 14, a shift of 100 mV in $\Delta G^0$ from the value for PhQ would not result in a significant change in the rate because of the broad maximum around the optimal value. This also means that with PhQ in the A1 site, the distribution of $\Delta G^0$ values required to produce an appreciable effect on the rate is very broad. In contrast, with AQ in the A1 site, a narrower distribution can extend into the steep region of the Marcus curve, leading to dramatically lower rates for a fraction of the reaction centers. Hence, a relatively narrow distribution of $\Delta G^0$ values could lead to the broad distribution of rates proposed to explain the temperature dependence of the polarization patterns. Such a broad distribution would also reconcile the optical (14) and EPR data (this work), which show picosecond and nanosecond $A_0^*$ lifetimes, respectively, for acceptors with midpoint potentials similar to AQ.

As discussed with Fig. 4, the major (slow) component of ET from PhQ$^-$ to F$_X$ in native PS I is known to have a significant activation energy and is therefore not at the maximum of the Marcus curve (i.e. $-\Delta G^0 = \lambda$). One way to understand the faster rates observed for AQ is as resulting from a lowering of the activation energy due to the more negative midpoint potential for AQ. In this case, the slope of the Arrhenius plot in Fig. 4 can be used to determine activation energy from which $\Delta G^0$ for the AQ$^-$ to F$_X$ ET step can be estimated. However, because the activation energy is dominated by $\lambda$, the slope is not very sensitive to a change in a small $\Delta G^0$, and the error in the value obtained would be relatively large. Moreover, careful examination of Fig. 4 shows that the slope of the AQ data is probably greater above 240 K than below (see the legend to Fig. 4). Such behavior is not surprising, since PS I in aqueous solution undergoes a transition to a disordered state at approximately this temperature (57), depending on the composition of the medium. For native PS I, it is not clear whether a change in slope occurs below the glass point, because there are no optical or EPR data available for this region. (Note that for optical studies, glycerol/water mixtures are preferred because the glass transition occurs at lower temperature. The glass point $T_g$ is around 190 K for a 50/50 mixture and is lower for higher glycerol content.) For AQ, the lower slope below 240 K is consistent with the expectation that the reorganization energy should be lower in a more rigid medium. Because the number of data points above 240 K is small and they have a large error, we have chosen not to use the slope from Fig. 4 to determine $\Delta G^0$. Rather, we have calculated the change in $\Delta G^0$ compared with native PS I using the absolute rates for selected temperatures. We then confirm that the predicted activation energy is consistent with the data. To calculate the absolute rates, we require the optimal rate. Based on reasonable parameter ranges (see Table II), the experimentally determined $A_0$ to F$_X$ rate of (250 ns)$^{-1}$ at room temperature ($\sim 295$ K) and the activation energy of $E_{act} = 220 \pm 20$ meV, the optimal rate has been evaluated to $2 \cdot 10^{10}$ s$^{-1}$ (57). Note that this result does not depend strongly on the value of $\Delta G^0$, since it is small compared with $\lambda$ (0.8 to 1.1 eV). If an exponential dependence of the electronic coupling on distance is assumed, the optimal rate corresponds to an edge-to-edge distance $R \sim 8 \text{ Å}$, which is in satisfactory agreement with the distance evaluated from the PS I x-ray structure (see Ref. 1 and Protein Data Bank entry 1JBO). Then, using Equation 2 and the values for $\lambda$ and $V$ given in Table II, we can calculate the value of $\Delta G^0$ required to reproduce the observed rates for the AQ to F$_X$ ET step between 240 and 260 K. This calculation predicts that with AQ in the A1 site, $\Delta G^0$ should be $-240 \pm 10$ meV between AQ and F$_X$, in good agreement with the expected value of approximately $-200$ meV based on the difference in reduction midpoint potentials of AQ and PhQ (15). With the range of values for $\lambda$ given in Table II, the estimate of $\Delta G^0$ leads to an activation energy in the range of 80–180 meV, which is compatible with the slope of the data above 240 K in Fig. 4 (see legend to Fig. 4).

In summary, the kinetic behavior can be rationalized in terms of classical Marcus theory and a shift of the quinone redox potential by about $-100$ meV. However, we note that a change in rate without an accompanying change in the activation energy, as encompassed by more sophisticated models of electron transfer (69, 70), is not excluded by the data. Investigation of a series of substituted quinones in which the midpoint potential of the quinone is varied stepwise over a wider range is needed to clarify the correlation between the absolute rates, activation energy, and midpoint potential. Experiments of this type are under way.

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