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Electron transfer through the quinone acceptor in cyanobacterial Photosystem I

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Introduction

In this article, we focus on the issue of whether electron transfer is unidirectional along one branch or bidirectional along both branches (*i.e.* through Q_K-A and Q_K-B) of the symmetrically-located electron acceptors in Photosystem (PS) I. The problem of whether electron transfer is unidirectional or bidirectional has its origins in the pseudo-C₂ symmetric configuration of the electron transfer cofactors. This issue cannot be addressed by X-ray crystallography and can only be determined using dynamic, *i.e.* spectroscopic, methods. In PS I there is no *a priori* reason to believe that electron transfer must be either uni- or bi-directional. Indeed, there is no obvious need for one-electron Q_A-type and for a 2-electron Q_B-type quinone as in PS II. Hence, a bidirectional pathway of electron transfer can easily be envisioned. Alternately, the 2.5 Å electron density map of PS I shows that there are subtle but important differences in the distances, orientations, and environments of the cofactors along both branches, and since rates of electron transfer are sensitive to these parameters, the probability that both branches are equivalent is negligible. Thus, a unidirectional pathway of electron transfer can be equally envisioned. Spectroscopic indicators have so far failed to show any evidence for directionality of electron transfer in PS I as in the case of the bacterial reaction centers, in which the two pheophytins can be distinguished on the basis of their optical properties.

The few experimental studies on the issue of directionality are thus far mutually incompatible. Evidence for unidirectionality is based on the photoaccumulation of A₀⁻ generated in lieu of A₁⁻ in PsaE and PsaE/PsaF deletion mutants of *Synechococcus* sp. PCC 7002 (Yang et al., 1998). Since the accumulation of A₀⁻ can occur only when the quinone is doubly reduced, a necessary precondition must be that the semiquinone anion becomes protonated prior to the second reduction step. Protonation can only occur if a water channel is available to the carbonyl group of the quinone, and this is proposed to result as a consequence of the removal of PsaF and the subsequent action of Triton X-100. On one extreme, if electron transfer were strictly unidirectional, then there should have been either 0% or 100% loss of the PhQ⁻ signal depending on which branch is active. On the other extreme, if electron transfer were strictly bidirectional, then there should have been a loss of exactly 50% of the PhQ⁻ signal. The total loss of photoaccumulated A₁⁻ and its replacement by A₀⁻ in the PsaE and PsaE/PsaF mutants thus implies that electron transfer is unidirectional along the quinone is associated

with the nonprimed α -helices (now assigned to PsaA) on the electron density map. Evidence for bidirectionality is based on an explanation of the long-known biphasic kinetics of PhQ⁻ oxidation as due to electron transfer up the two different branches of cofactors in PS I (Joliot & Joliot, 1999). This interpretation is supported by mutagenesis studies that involved changing the π -stacked Trp on the PsaA- and PsaB-subunits of *Chlamydomonas reinhardtii*. A Trp->Phe mutation on the PsaB-side slowed the 18 ns kinetic phase to 97 ns, and a Trp->Phe mutation on the PsaA-side slowed the 218 ns kinetic phase to 609 ns (Guergova-Kuras et al., 2001). Since only the kinetics were changed, and not the amplitudes of the two kinetic phases, this result implies that electron transfer is bidirectional in PS I. An independent EPR study of PsaA-side Trp->Leu and Trp->His mutations in *C. reinhardtii* showed a slowing of forward electron transfer as measured by the decay of the electron spin-polarized signal arising from the P700⁺A₁⁻ radical pair (unfortunately, there are no results reported on PsaB-side mutants) (Purton et al., 2001). While the issue of uni- and bi-directionality issue remains unresolved among these three experiments, there is agreement on one point: the slow kinetic phase of electron transfer occurs on the quinone associated with the PsaA subunit. The uncertainties include: does the optical kinetic spectroscopy measured in whole cells reflect the same chemistry as the photoaccumulated and electron spin polarized EPR signals measured in PS I complexes; does the pathway differ depending on whether the organism is a prokaryotic cyanobacteria or a eukaryotic plant; and does the isolation of the PS I complex alter the pathway up the two near-symmetrical branches of electron acceptors?

We approached this problem by studying site-directed mutants separately in the Q_k-A and the Q_k-B binding sites on PsaA and PsaB in *Synechocystis* sp. PCC 6803. Our reasoning is that any change in the redox potential would be expected to translate to a change in the forward as well as backward kinetics of electron transfer to and from the altered quinone. The initial planning of these experiments required more structural knowledge of the quinone binding sites than was available in the 4 Å model of PS I (Klukas et al., 1999). This

information was provided by EPR and ENDOR studies that indicated an asymmetric H-bond from an unidentified amino acid, and a Trp in van der Waals contact with PhQ (Hanley et al., 1997), most probably π - π stacked (Iwaki & Itoh, 1991). The π - π -stacked Trp, a H-bond from the PhQ carbonyl (ring position 1, adjacent to the methyl group) to a backbone amide from L722_{PsaA} and L706_{PsaB}, and the absence of a H-bond to a second PhQ carbonyl, have recently been confirmed in the 2.5 Å model of PS I (Jordan et al., 2001). Alteration of the H-bond to the quinone carbonyl will be difficult, since it is to an amino acid peptide backbone rather than to a side chain. However, the Trp in π - π contact with the quinone (see Figure 1) is an excellent

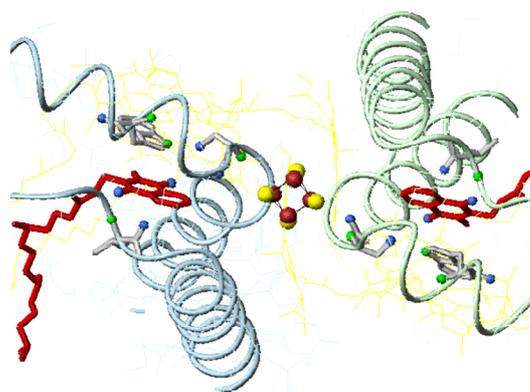


Figure 1. Depiction of the quinone binding sites on the PsaA-side and PsaB-side of PS I. From: 1JB0

candidate; furthermore, it is likely involved in modulating the redox properties to the quinone. Interestingly, a side chain oxygen of a serine residue is a little over 3 Å distant from the second PhQ carbonyl group on both the PsaA and PsaB sides (ring position 4, adjacent to the phytol chain). However, S692_{PsaA} and S672_{PsaB} are H-bonded to the imidazole nitrogen of the Trp residues that are in π - π contact with the quinones. While the distance and the bond angle between the serine and the quinone argue against a H-bond between these two molecules, we reasoned that the Ser residues stabilize the Trp in π - π contact with the quinone. The Ser residues were therefore considered a second excellent candidate for mutagenesis.

Results and Discussion

Photoaccumulated CW EPR Spectrum at Q-Band: W697F_{PsaA} and W677F_{PsaB}

The photoaccumulated EPR spectra of A_1^- in the wild-type and W697F_{PsaA} and W677F_{PsaB} mutants are depicted in Figure 2. The Trp residues 697 on PsaA and 677 on PsaB are π - π stacked with the quinones, and are nearly equivalent on both subunits with respect to distance between the ring systems and the mutual orientation of the rings. At 34 GHz (Q-band), the g -anisotropy dominates the EPR spectrum of A_1^- in wild-type PS I, allowing the $g_{xx} = 2.0062$ and $g_{zz} = 2.0021$ components of the tensor to be resolved. The four prominent hyperfine lines from the -CH₃ fragment obscure the g_{yy} component of the tensor, and result from the high spin density at the carbon position 2 of the phyllosemiquinone anion radical with the methyl group attached. A small amount of A_0^- is present, and contributes to the overall spectrum in the highfield (g_{zz}) region. The W677F_{PsaB} mutant was found to be similar to the wild-type, showing clearly resolved hyperfine couplings and a relatively small contribution of A_0^- . The spectrum of the W697F_{PsaA} mutant is also similar to the wild-type, with prominent hyperfine couplings but a much greater contribution of A_0^- .

Photoaccumulated CW EPR Spectrum at Q-Band: S692C_{PsaA} and S672C_{PsaB}

The photoaccumulated EPR spectra of A_1^- in the S692C_{PsaA} and S672C_{PsaB} mutants are depicted in Figure 4. The Ser residue 692 on PsaA is 3.27 Å

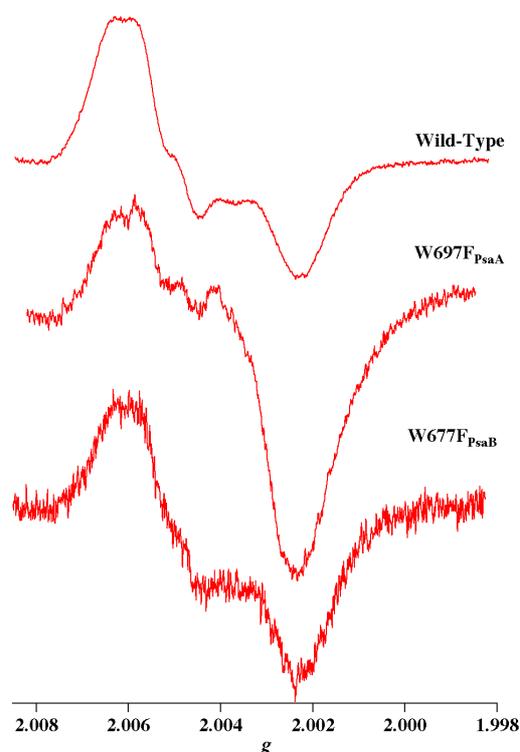


Figure 2. Photoaccumulated CW EPR spectra of wild-type, W697F_{PsaA} and W677F_{PsaB} mutants.

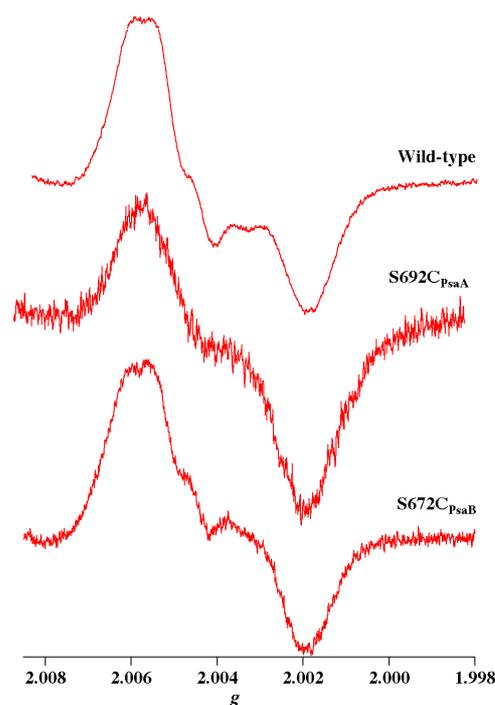


Figure 3. Photoaccumulated CW EPR spectra of wild-type, S692C_{PsaA} and S672C_{PsaB} mutants.

Q_K -A and Ser residue 672 on PsaB is 3.37 Å distant from the carbonyl at position 4 of Q_K -B. Although the distances and bond angles argue against the presence of a H-bond, the substitution of a cysteine for a serine might be expected to alter the properties of the quinone by virtue of the H-bond to W697F_{PsaA} and W677F_{PsaB}. The spectrum of the S672C_{PsaB} mutant was found to be identical with the wild-type, showing clearly resolved hyperfine couplings and a relatively small contribution of A_0^- . The spectrum of the S692C_{PsaA} mutant, however, has a linewidth equivalent to that of the wild-type, and there is conspicuous absence of prominent hyperfine lines along with a slightly greater contribution from A_0^- .

W693F_{PsaA} and W667F_{PsaB}: Electron spin polarized EPR spectra at X-Band

Figure 4 shows room temperature decay associated spectra (left) obtained by a global fit of kinetic transients. The single transient shown (right) is recorded at 3481 G. The decay associated spectra have been extracted from the complete time/field data sets. Positive signals correspond to absorption (A) and negative signals correspond to emission (E). As the electron is transferred to the iron sulfur clusters, the initial E/A/E polarization pattern due to $P^+A_1^-$ changes to the

primarily emissive P^+ contribution to the P^+FeS^- spectrum. This transition is seen clearly in the transients on the right. In the top two panels PS I from the PsaA mutant is compared with the wild type while in the bottom two panels the same comparison is made for the PsaB mutant. Clearly, the mutation in PsaA leads to a slowing of the rate of electron transfer and a change in the spectra while no difference between the PsaB mutant data and wild type data is detectable within experimental error. An initial fit of the data yields a lifetime for the electron transfer of $\tau = 240 \pm 50$ ns in the wild type and PsaB mutant while in the PsaA mutant it is slowed to $\tau = 520 \pm 50$ ns. As can be seen in the comparison of the spectra of the wild type and PsaA mutant the hyperfine structure due to the methyl group adjacent to the phytol tail of phyloquinone is more pronounced in the spectrum of the PsaA mutant. Simulations

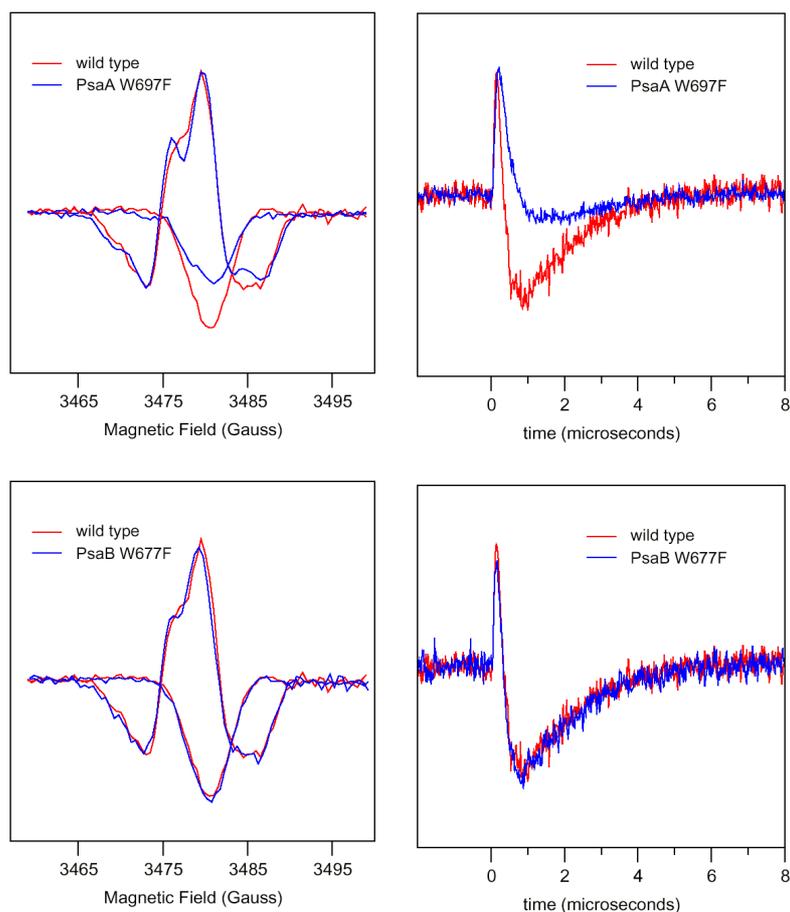


Figure 4. Transient EPR spectra (left) and kinetics (right) of wild-type (red), W697F_{PsaA} (top) and W677F_{PsaB} (bottom) mutants.

of the spectra (not shown) indicate that the strength of the hyperfine coupling does not change but that the residual linewidth is narrower in the spectrum of the mutant. This result is likely due to a change in the hyperfine couplings from the protein when Trp 693 on PsaA is changed to Phe.

S692C_{PsaA} and S672C_{PsaB}: Electron spin polarized EPR spectra at X-band.

The room temperature transient EPR spectra of P700⁺ A₁⁻ in the wild-type and the S692C_{PsaA} and S672C_{PsaB} mutants are depicted in Figure 5. In the S672_{PsaB} mutant, $\tau = 220 \text{ ns} \pm 50 \text{ ns}$, but in the S692C_{PsaA} mutant, $\tau = 920 \pm 50 \text{ ns}$, which is even slower than the W693F_{PsaA} mutant.

Thus, forward electron transfer is slowed in the mutants by a factor of ca. 4. The early spectrum in the S692C_{PsaA} mutant has the same *g*-anisotropy as the wild-type, but a less pronounced hyperfine pattern, as shown by the loss of features around 3477 G and 3485 G. The late spectrum also shows a lower contribution from P700⁺, consistent with

the much diminished emissive contribution around 1 μs in the kinetic spectrum. In contrast, the kinetics as well as the early and late spectrum in the W677F_{PsaB} mutant are indistinguishable from the wild-type.

Although the spin polarization patterns are sensitive to spin dynamics on a timescale faster than the instrumental rise time, fast electron transfer through the PsaB pathway cannot be rigorously excluded on the basis of these data alone.

Slowing of A₁⁻ Oxidation at 260 K

When a wild-type PS I complex is cooled to a temperature of 260 K, charge separation between P700 and F_A/F_B remains reversible, yet forward electron transfer slows by a factor of ca. three. Since the same holds true of the W693F_{PsaA} and S692C_{PsaB} mutants, our expectation is that the slowing of electron transfer on the

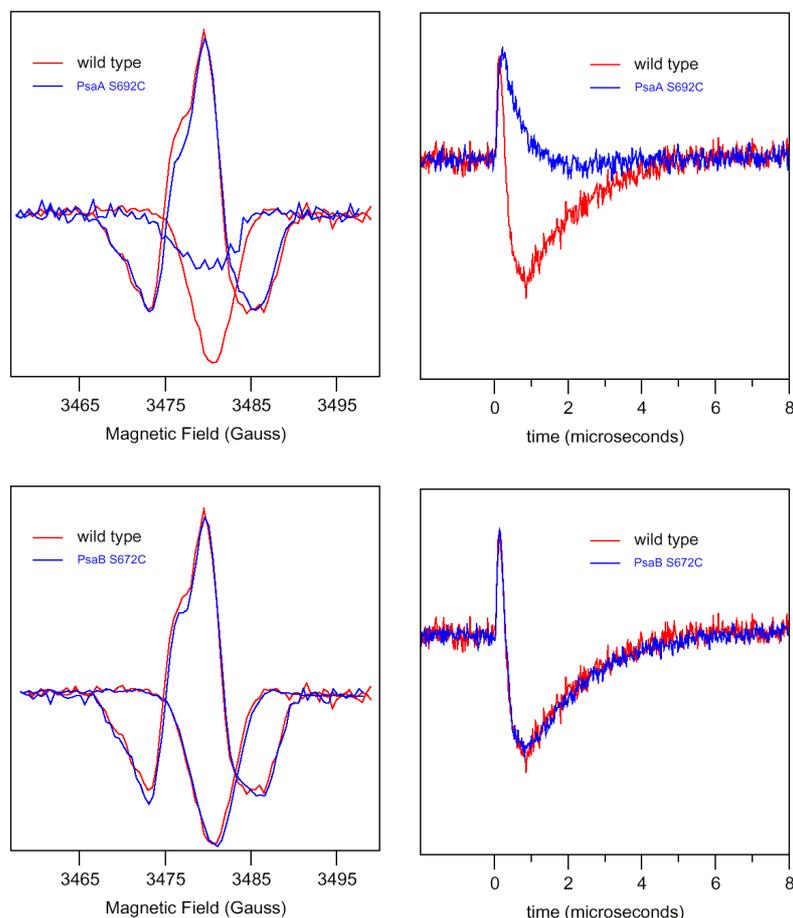


Figure 5. Transient EPR spectra (left) and kinetics (right) of wild-type (red), S692C_{PsaA} (top) and S672C_{PsaB} (bottom) mutants.

PsaB side will be similar. Nevertheless, the kinetics of A_1^- oxidation in the $W673F_{PsaB}$ and $S672C_{PsaB}$ mutants remain identical to the wild-type at 260 K, and show no indication of a fast kinetic phase. If, as determined by optical kinetic spectroscopy, the $\tau(1/e)$ of the fast phase is ca. 10 ns in *Synechocystis* sp. PCC 6803 (Brettel, 1998), then the effect of the Ser->Cys mutation and the low temperature should have slowed the kinetics to ≥ 100 ns, which is within the window of detection by transient EPR spectroscopy.

Optical kinetic data from *C. reinhardtii* shows evidence for A_1^- oxidation along the PsaB-side with $\tau(1/e)$ of >10 ns in the wild type and correspondingly slower kinetics in the Trp->Phe PsaB-side mutant (Guergova-Kuras et al., 2001). We performed kinetic measurements at 260 K with improved time resolution (<50 ns) and with help from a characteristic short time spin dynamics effect.

Figure 6 depicts a set of fast kinetics for the same samples used in Figure 5 but with an expanded time scale and for seven (0.75 G) field steps between the maximum absorptive signal of the early $P700^+ A_1^-$ spectrum and the up-field emissive minimum, *i.e.* through the up field zero-crossing of the signal (see Figure 5 left). We emphasize in particular the following observation. Nearly all transients show an initial absorptive peak even when the signal after ca. 100 ns is already emissive, *i.e.* the field positions are already up-field of the zero-crossing of the ‘early’ spectrum due to the $P700^+ A_1^-$ radical pair. The effect is readily explained by a characteristic spectral broadening effect at early times after the exciting laser pulse. The suddenly-generated $P700^+ A_1^-$ radical pair spins experience the microwave field as suddenly turned on with correspondingly increased excitation bandwidth. Hence, the initial spectral broadening shifts the zero-crossing of the spin polarized $P700^+ A_1^-$ spectrum to higher fields. Therefore, the initial absorptive kinetic peak is also characteristic for the $P700^+ A_1^-$ radical pair state which would be reduced if electron transfer past A_1^- would be fast. (Note a clear indication of signal modulation on the early decay slope of the transients in Figure 6. These demonstrate the existence of ‘nuclear coherences’ first observed in bacterial reaction centers (Bittl et al., 1994; Weber et al., 1995).

The results of Figure 6 demonstrate the same fast kinetic behavior for wild type as well as for both serine mutants (with their distinctly different electron transfer kinetics along the PsaA side). Thus, for cyanobacterial PS I complexes there is no indication from EPR spectroscopy for the existence of a significant contribution ($>10\%$) of a fast kinetic phase (equal or longer than ca. 20 ns) along the PsaB side.

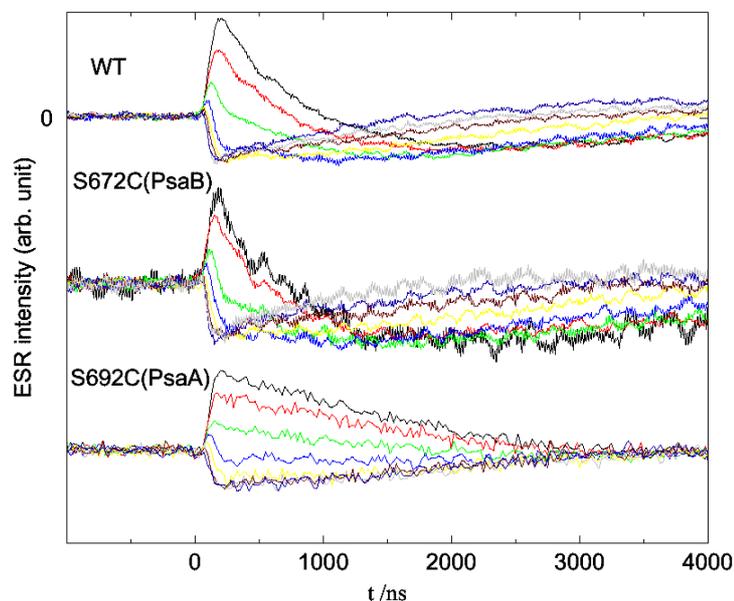


Figure 6. Transient EPR kinetics of wild-type (top), $S672C_{PsaB}$ (middle) and $S692C_{PsaA}$ (bottom) mutants at 260 K.

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