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Mutational analysis of the binding sites of the phylloquinones in PS I

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Introduction

Photosystem I (PS1) is a multi-subunit thylakoid membrane protein that converts electromagnetic energy into chemical energy using photons to drive electron transfer across the membrane. The core of PS1 is a heterodimer of the related PsaA and PsaB subunits, each containing 11 transmembrane α-helices. The first 6 helices bind chlorophyll (Chl) molecules of the core antenna, and the last 5 helices hold the cofactors involved in electron transfer.

Charge separation produces a spin-polarized EPR signal that decays in ns to μs, depending upon temperature (Bock et al., 1989). An EPR signal with similar *g*-value and line-width to quinones can be accumulated by illumination at low temperature after reduction of Fe-S clusters. The high-field EPR (MacMillan et al., 1997) and ENDOR spectra of photo-accumulated A_1^- (Teutloff et al., 1998), as well as the conformity of hyperfine couplings (hfc's) measured in photo-accumulated and transient A_1^- (Bittl et al., 1998), allowed the conclusion that A_1^- originates from a unique quinone. ESEEM indicated that a Trp and perhaps another N-containing aromatic group were nearby (Hanley et al., 1997). EPR analysis of photo-accumulated A_1^- in oriented PS1 (MacMillan et al., 1997) and of the P_{700}^+ A_1^- radical pair in PS1 crystals allowed prediction of the phylloquinone sites in the three-dimensional structure (Bittl et al., 1997); electron densities in these regions were assigned to the two phylloquinones (Klukas et al., 1999) in an area where the tenth transmembrane α-helices of PsaA and PsaB are linked to an α-helix parallel to the membrane plane.

Materials and methods

Site-directed mutations were made using a system for mutagenesis of *Chlamydomonas* PsaA and PsaB (Redding et al., 1998). Thylakoid membranes were prepared and immunoblotting was performed as described (Redding et al., 1998). A_1^- was photo-accumulated in membranes by reduction with sodium dithionite at pH 8 for 30 minutes in the dark at room temperature, illumination for 30 minutes at 200 K, and incubation in the dark at 200 K for 5 minutes. Q-band cw-EPR spectra were measured on a Bruker E-500 spectrometer at 80 K in the dark (microwave frequency = 34.00 GHz, power = 6 μW, modulation frequency = 10 kHz, modulation amplitude = 3.5 G_{pp}.). TR-EPR was done as described (Bittl and Zech, 1997), except weak selective 90° and 180° pulses were used to record the field-swept echo-detected spectra with the following sequence: laser flash - t_1 - 90° microwave pulse - t_2 - 180° pulse - t_2 - echo. Time-resolved ENDOR was performed as described (Bittl et al., 1998).

Results

Site-directed mutagenesis was used to survey the PsaA and PsaB polypeptides for residues that form part of the phylloquinone-binding sites. An extended region from the middle of helix 10 to the middle of helix 11 was targeted, as this region would be nearest to the electron densities assigned to phylloquinones in the 4-Å crystal structure (Klukas et al., 1999), and it is analogous to where quinones are bound in the bacterial type 2 reaction center. This resulted in four pairs of targets: Trp693/673, Trp702/682, His704/684, and His730/714. We constructed double mutants, which harbored mutations of both members of the pair.

To determine whether any of the mutations cause the loss of one or more subunits, quantitative immunoblots were performed with antibodies raised against PsaA, PsaC, PsaD, PsaE (not shown), and PsaF. Although all of the double mutants had a lower level of PS1 in their thylakoid membranes (~50% of WT), each of the subunits was present roughly in the same stoichiometry as in WT (Fig. 1).

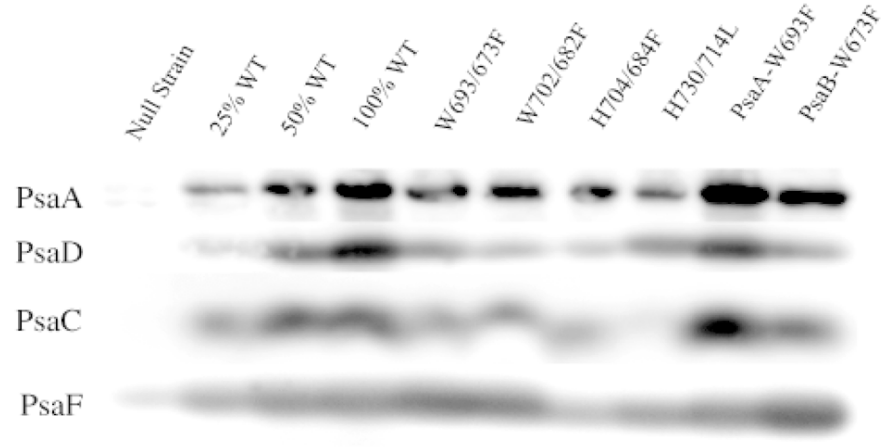

Figure 1: Immunoblots of thylakoids (0.5 µg Chl/lane) from indicated strains.

Q-band EPR spectra of photo-accumulated A_1^- radical are shown in Figure 2. Although A_0^- is present, these EPR signals can be partially resolved at Q-band frequencies (34 GHz). An A_1^- EPR signal was observed in all double mutants except W693F/W673F after 10 minutes illumination at pH 10 and 200K (data not shown). When the photo-accumulation was repeated at pH 8.0, it was possible to photo-accumulate a broad EPR signal resembling a mixture of A_0^- + A_1 in the W693F/W673F mutant at pH 8 (Fig. 2). A symmetric spectrum that appeared to arise strictly from A_0^- was observed in the PsaA-W693F single mutant (Fig. 2). In the PsaB-W673F single mutant, however, a high A_1^-:A_0^- ratio was reproducibly observed (Fig. 2). Thus, the point mutation PsaA-W693F has a specific effect on the ability to photo-accumulate the A_1^- radical.

Time-resolved X-band pulsed EPR spectroscopy was performed at 80K on thylakoid membranes. $P_{700}^+A_1^-$ spectra were observed in the WT, single mutants (PsaA-W693F and PsaB-W673F), and the double mutant (Fig. 3). Both the PsaA-W693F mutant and the double mutant exhibited an alteration in a partially-resolved hyperfine structure, which is observable as a shoulder on the low-field side of the main peak in this spectrum. This feature, which is much more distinct in cyanobacterial PS1, has been attributed primarily to hyperfine couplings from the 2-methyl protons of phylloquinone (Bittl et al., 1998). The specific effect of the PsaA-W693F mutation upon the hfc's of A_1^- demonstrates that PsaA-Trp693 is close to the phylloquinone normally involved in reversible charge separation at low temperature.

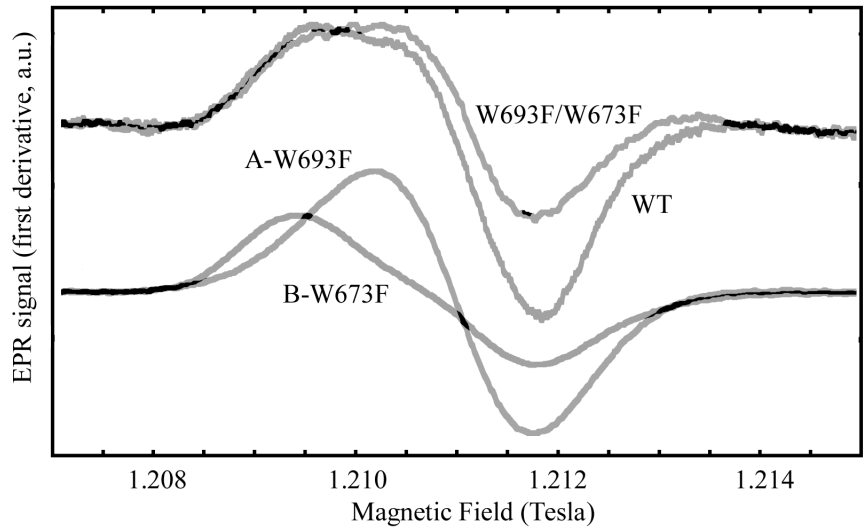

Figure 2: Q-band EPR spectra of photo-accumulated A_1^- in thylakoid membranes.

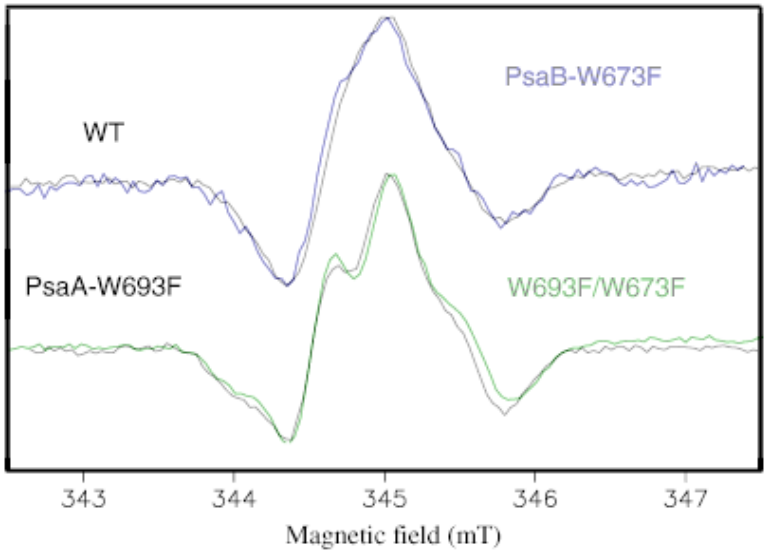

Figure 3: Time-resolved EPR spectra of P_{700}^+ A_1^- in PS1 from the indicated strains.

It might be that in the PsaA-W693F mutant, the hyperfine couplings of the methyl protons themselves or other couplings have shifted, allowing a better resolution of this feature. An interesting possibility is that the removal of the indole nitrogen by mutation removes a ^{14}N coupling to the phylloquinone radical. The latter interpretation is compatible with the fact that the time-resolved proton ENDOR spectrum of the radical pair in the PsaA-W693F mutant closely resembles that of the wild type (Fig. 4).

Discussion

We conclude that the indole of PsaA-Trp693 is part of the A_1 binding site, which agrees perfectly with the new 2.5-Å crystal structure of cyanobacterial PS1 (Jordan et al., 2001). The tryptophan shown here to affect the A_1^- EPR signal π-π stacks with the phylloquinone bound to PsaA, while the symmetry-related phylloquinone is similarly stacked with the analogous tryptophan in PsaB (Jordan et al., 2001). Using these same mutants, optical evidence has

recently been obtained for bi-directional electron transfer *in vivo* (Guergova-Kuras et al., 2001). The reason for the apparent discrepancy between the EPR data and the *in vivo* optical data is unknown. However, we note that there is a difference in the EPR spectra between WT and PsaB-W673F and between PsaA-W673F and the W693F/W673F double mutant. These differences may be due to the phylloquinone on the PsaB side. This hypothesis is being tested by several experiments, including high-field EPR measurements on these mutants.

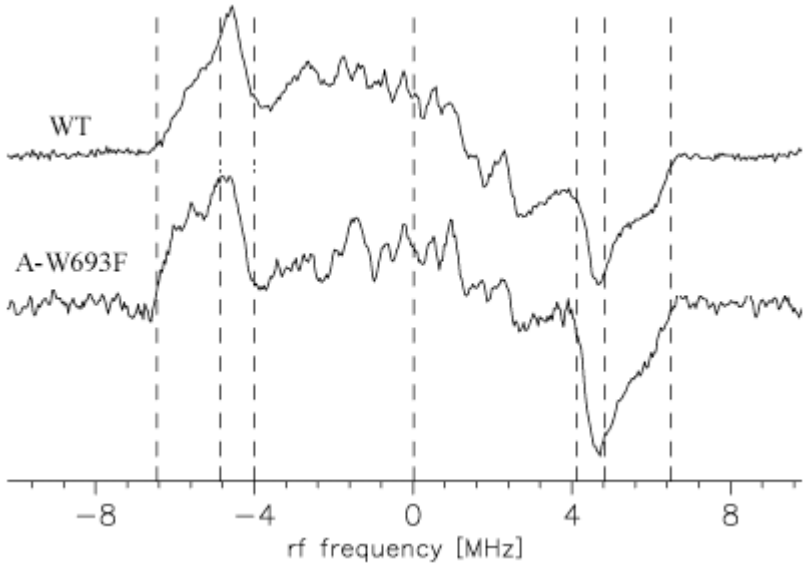

Figure 4: Time-resolved ENDOR spectra of WT and PsaA-W693F mutant PS1.

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