Final report

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Recipient Institution: The University of Alabama

Project Title: A Combined Genetic, Biochemical, and Biophysical Analysis of the A₁ Phylloquinone Binding Site of Photosystem I from Green Algae

Principal Investigator: Kevin Redding

Distribution limitations: none

Executive summary of project accomplishments

This project has resulted in the increase in our understanding of how proteins interact with and influence the properties of bound cofactors. This information is important for several reasons, including providing essential information for the re-engineering of biological molecules, such as proteins, for either improved function or entirely new ones. In particular, we have found that a molecule, such as the phylloquinone used in Photosystem I (PS1), can be made a stronger electron donor by placing it in a hydrophobic (greasy) environment surrounded by negative charges. In addition, the protein is constrained in its interactions with the phylloquinone, in that it must bind the cofactor tightly, but not in such a way that would stabilize the reduced (natively-charged) version of the molecule. We have used a combination of molecular genetics, in order to make specific mutations in the region of the phylloquinone, and an advanced form of spectroscopy capable of monitoring the transfer of electrons within PS1 using living cells as the material. This approach turned out to produce a significant savings in time and supplies, as it allowed us to focus quickly on the mutants that produced interesting effects, without having to go through laborious purification of the affected proteins. We followed up selected mutants using other spectroscopic techniques in order to gain more specialized information.

Summary of proposed project

This project focuses on the phylloquinone (PhQ) cofactor of PS1. The primary electron donor (P_{700} , a pair of chlorophylls) is connected to the tertiary electron acceptor (F_X , an Fe_4S_4 cluster) by two branches of cofactors. Each branch contains a pair of chlorophylls (ec2 and ec3) and a PhQ (see Fig. 1). These cofactors are bound by two related membrane polypeptides, PsaA and PsaB, in a pseudo- C_2 -symmetrical structure. We proposed to use a combination of site-directed mutagenesis and spectroscopic analysis in order to understand better how the PsaA/PsaB polypeptides of PS1 modulate the properties of the PhQ cofactor. The following hypotheses were listed in the grant proposal as the primary ones that we would test:

- 1) Both branches of cofactors are active, and each PhQ plays a role in reducing F_X .
- 2) The redox properties of the PhQs are due to interaction with residues in PsaA/B.
 - a. These provide them with a hydrophobic environment.
 - b. Nearby (negative) charges lower the PhQ's midpoint redox potential.
 - c. Some residues are involved as H-bond donors to the quinone oxygen(s).
- 3) A corollary to the above hypothesis is that differences in the properties of the two PhQs must be due to differences in their environments.

The specific aims of this project were to

- 1) Modify the character of the PhQ niche
- 2) Reduce nearby negative charges
- 3) Evict PhQs from specific sites
- 4) Identify "chlamyquinone" (the quinone used in PS1 by *Chlamydomonas reinhardtii*)
- 5) Perform site-specific reconstitution of tail-channel mutants with tail-less quinones
- 6) Make combinations of mutations to test specific hypotheses

Figure 1. Arrangement of the core cofactors of Photosystem I. The pseudo- C_2 symmetry axis is shown as a dotted gray line dividing the two branches. For sake of clarity, the phytol chains of the cofactors have been truncated and the terminal acceptors F_A and F_B are not shown. Reduction of the ec3 Chl(s) occurs in <100 ps, with subsequent electron transfer to the PhQ on a similar timescale. In the nanosecond timescale, the electron is transferred to F_X and then to F_A/F_B . Our main working model is that ET can proceed down either the A-branch or B-branch, leading subsequently to ET from either PhQ_A or PhQ_B with slower or faster kinetics, respectively.

PsaA-Y696F: increases 20-ns/200-ns amplitude ratio (PhQ^- reoxidation)

PsaA-W697F: slows 200-ns phase

PsaA-S692A: slows 200-ns phase

PsaA-G693W: slows 200-ns phase

PsaA-F689W: slows 200-ns phase somewhat

PsaA-F689N: slows 200-ns phase severely

PsaA-L722T: accelerates 200-ns phase

PsaA-L722Y: decreases amplitude of 200-ns phase and shows signs of back-reaction

Figure 2. The PhQ_A site, based on the crystal structure of PS1 from *Thermosynechococcus elongatus* (Jordan et al, 2001). Important amino acid residues mentioned in the text are shown, along with a summary of the effects of various mutations made in the course of this project. Note that the numbering used here and throughout the text follows that of *T. elongatus*.

Accomplishments

Combining mutations that alter directionality of ET within PS1 with those that slow reoxidation of PhQ_A (hypothesis 1 & aim 6): We have combined two of the mutants that slow ET from PhQ_A (PsaA-W697F and PsaA-S692A) with one that redirects ET from the A-branch to the B-branch (PsaA-Y696F; Li et al, 2006). The primary reason for doing this was to test our interpretation of the ET kinetics even further. We had a very specific prediction for this

combination of mutations: the effects of the mutations would be additive. That is, we would see both the change in amplitudes seen in the PsaA-Y696F mutant, and the slowing of the kinetic component attributed to PhQ_A^- reoxidation seen in the PhQ_A site mutants. As can be seen in Figure 3, this prediction is met in both the PsaA-S692A/Y696F and the PsaA-Y696F/W697F double mutants. This work has just been submitted for publication to *Photochemistry and Photobiology* (see attached reprint).

These results have the exciting implication that it is possible to control independently the yield of reduction of each PhQ (*i.e.* directionality) and their rate of reoxidation. Although this was implied from the previous work on the single mutants, the results from the double mutants demonstrates that this can be done *simultaneously*. In principle, we had no right to expect this with these specific combinations of mutations, given how close they are to each other in the primary and tertiary structure of PS1. The close convergence of the changes in rates and amplitudes seen in the single mutants and double mutants, however, demonstrates just how independent their effects are. Thus, the structure of PS1 may be robust enough to accommodate multiple mutations without experiencing extensive interference from synergistic effects. Although it may be hazardous to over-generalize this phenomenon, these results at least open the possibility of re-engineering the internal working of PS1 in a controlled manner.

Figure 3. Pump-probe spectroscopic characterization of single and double mutant PS1 *in vivo*. Semi-log plot in ns- μ s timescale of absorption decay at 380 nm, which corresponds to reoxidation of PhQ_B^- (usually ~ 20 ns) and PhQ_A^- (usually ~ 200 ns) (Guergova-Kuras et al, 2001). Panel A: comparison of PsaA-Y696F (diamonds), PsaA-W697F (squares), and PsaA-Y696F/W697F (circles). Panel B: comparison of PsaA-Y696F (diamonds), PsaA-S692A (squares), PsaA-S692A/Y696F (circles).

Properties of the two PhQ-binding sites (hypotheses 2 and 3): One of our central hypotheses is that the hydrophobic environment of the PhQ niche is key to making it so reducing. We have made mutations of the key hydrophobic residues that surround the 2-methylnaphthoquinone headgroup of PhQ_A :

- 1) PsaA-Trp693: to Phe (our original mutation), Leu, Ala
- 2) PsaA-Phe685: to Trp, His, Asn
- 3) PsaA-Leu718: to Thr, Tyr, Trp

Some of these (PsaA-W697A, PsaA-L722W) did not accumulate PS1, which is a common occurrence and likely related to problems with assembly. We have been able to obtain data with the others, some of which are shown below in Figure 4.

Figure 4. Decay of absorption at 480 nm in ns- μ s timescale as a semi-log plot. This wavelength corresponds to bandshifts of pigments near the PhQs, as well as P_{700}^+ ; thus, it monitors reoxidation of PhQ_B^- (usually ~ 20 ns) and PhQ_A^- (usually ~ 200 ns) and re-reduction of P_{700}^+ (usually ~ 5 μ s) (Guergova-Kuras et al, 2001). Decay kinetics are presented for WT (black squares), and the mutants PsaA-L722T (green triangles), PsaA-F689W (red circles), and PsaA-F689N (blue diamonds).

We have two especially exciting results here. The first is that we have a new record for the effect of a mutation upon the rate of ET from PhQ_A to F_X – the PsaA-F689N mutation results in a 100-fold retardation. In this mutant, the $PhQ_A \rightarrow F_X$ step is the slowest one in the complex, which is a unique situation. We plan to use this mutant to test the hypothesis that it would be possible to double-reduce the quinone when a second electron is sent down the system. The second discovery is the first mutation that actually *accelerates* this ET step: PsaA-L722T.

Eviction of PhQs (aims 3 and 5): As mentioned in the proposal, we have evidence that the PsaA-L722Y mutation causes eviction of the quinone from the PhQ_A site, due to steric clash between the Tyr and the phytyl chain of the quinone. These include: (1) lower amplitude of the ~ 200 -ns component; (2) altered DAS for the faster decay component; and (3) lowered amplitude of the 4- μ s decay component corresponding to re-reduction of P_{700}^+ (normalized to the initial bleaching). We have shown that the faster DAS is likely a superposition of the ~ 20 -ns decay component plus a new component corresponding to back-reaction from the $P_{700}^+ ec3_A^-$ state, which should take place in the 10-30 ns timescale. We have now purified PS1 from this mutant and the corresponding B-side mutation (PsaB-L706Y) and we have repeated these measurements with the particles. In addition, we have initiated a collaboration with Prof. John Golbeck (Penn. State Univ.), who has made similar mutants in the cyanobacterium, *Synechocystis sp.* PCC6803. Together, we have measured transient optical and transient EPR spectra of particles from mutants in both species. Both the optical (Fig. 5) and EPR (not shown) experiments are consistent with our hypothesis that the PsaA-L722Y mutation lowers the affinity of the PhQ_A site for phylloquinone, resulting in a certain fraction (20-40%) of centers lacking PhQ_A . The PsaB-L722Y mutations also produces noticeable effects (Fig. 5), which we do not yet understand.

We have also commenced experiments to see if addition of 2-methylnaphthoquinone can restore growth to the PsaA-L722Y mutant. Preliminary results indicate that it can (data not shown); we are now repeating this with a range of quinones. We have also started experiments in the nanosecond time scale with particles that have been “reconstituted” with 2-methylnaphthoquinone. Lastly, Dr Narasimhulu has tested these particles by cw-EPR at low temperature, to see if they can perform irreversible charge separation at 77 K, and found that they can.

Figure 5. Decay-associated spectra of fitted kinetic components in ns- μ s time scale for the PsaA-L722Y (panel A) and PsaA-L706Y (panel B) mutants. The former shows evidence of a back-reaction (bleaching at ~ 440 nm in ns components, plus the fact that the ~ 6 - μ s component due to P_{700}^+ reduction is lower than the initial bleaching at 430 nm). The latter seems to possess a single decay component

Abbreviations used:

Chl, chlorophyll; DAS, decay-associated spectrum/spectra; ELDOR, electron- electron double resonance; ENDOR, electron-nuclear double resonance; EPR, electron paramagnetic resonance; ET, electron transfer; F_x , an Fe-S cluster serving as tertiary electron acceptor; PhQ, phylloquinone; P_{700} , pair of chlorophylls serving as primary electron donor; TR, time-resolved

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Publications produced during the life of this grant

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