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Photosynthetic Electron Transport in Genetically Altered
Chloroplasts

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Abstract

We have generated and characterized the phenotypes of several photosystem II, D1 protein mutants in *Chlamydomonas reinhardtii* chloroplasts. The first set of mutants which we produced were at residue H195 on the D1 protein, adjacent to histidine -198 (H198), a putative ligand to the redox active chlorophyll special pair, P680. Conservative amino acid replacements at H195 had little effect on electron transfer whereas non-conservative replacements had significant effects on electron transfer processes, particularly rates of transfer from Z to P680. In contrast, histidine 190 (H190) mutants were unable to evolve oxygen, had reduced manganese content, slower rates of charge transfer from Z to P680 and lacked the A₁ thermoluminescence band. These results suggested that H190 may be a redox active histidine residue required for photoligation of manganese into the water oxidizing complex. EPR studies demonstrated that H190 does not H-bond to Z. This is in contrast to the H190 residue of the D2 protein which does H-bond to the redox active tyrosine, D. These are the first results which identify a specific histidine residue as potentially being redox active in photosystem II and have implications for the structure of the water oxidizing complex.

Using the chloroplast DNA co-transformation system developed in our lab we have generated three amino acid replacements at position H195 (histidine - 195) in the D1 protein of the photosystem II (PSII) reaction center complex. These mutants are: N195, Y195 and D195. The H195 residue was targeted for mutagenesis since it is near the putative chlorophyll special pair (P680) ligand, H198. The presence of a potentially positively charged residue near P680 was predicted to destabilize P680⁺ and facilitate its re-reduction. Measurements of rates of oxygen evolution in the H195 mutants indicated that the conservative replacement, N195 had little effect on the rate of oxygen evolution while the more non-conservative replacements reduced oxygen evolution by approximately 30%. This reduction in oxygen evolution was shown to be associated with a longer lived P680⁺ state as indicated by fluorescence induction and decay experiments in the presence and absence of an artificial electron donor. It was apparent, however, that H195 is not required for oxygen evolution. A second effect of mutagenesis of the H195 residue was alteration in the yield of the formation of the A₁ thermoluminescence band attributed to charge recombination between a redox active residue and reduced Q_A. Depending on the substitution, the yield of the A₁ band ranged from approximately 30-100% of wild type Mn depleted thylakoids. Interestingly, the mutations caused no shift in the temperature of the A₁ band indicating that there was no shift in the activation energy barrier.

A second site targeted for mutagenesis was the H190 residue on the D1 protein. This residue has been proposed to have several possible functions including: a Mn ligand of the water oxidizing complex, a redox active residue, H-bonding to the Z tyrosine and a ligand for an accessory chlorophyll. Three separate H190 mutants were made including an H190F mutant in a wild type and photosystem I minus strain and H190Y in a wild type strain. The phenotypes of these mutants were significantly different from wild type. None of the H190 mutants were able to evolve oxygen and each had a reduced manganese content (1Mn/250 Chl) relative to wild type (4Mn/250 Chl). The mutants were, however, able to oxidize artificial electron donors which reduce the redox active tyrosine Y_Z but at about half the maximal rate observed in Mn depleted wild type membranes. There was also a significant (100-1,000 fold) reduction in the time constant for the formation of Signal II_{last} from Y_Z.

There were two other noteworthy features of the H190 mutants. First of all, these mutants were unable to form the A₁ thermoluminescence band associated with oxidation of a redox active residue in Mn depleted membranes. The A₁ band has been attributed to charge recombination between Q_A⁻ and an oxidized histidine residue which may in turn oxidize Mn during its insertion into the S-state complex. Second, the mutants exhibited a normal Y_Z EPR signal. This is in contrast to H190 mutants on the D2 protein which have a significantly altered EPR signal associated with a redox active tyrosine, Y₁₁, located in a similar position as Y_Z in the D1 protein. These results suggest that the H190 residue in the D1 protein has a different environment than the corresponding H190 residue in the D2 protein and does not H-bond to the redox active tyrosine residue.

Since the H190 mutants had no Mn complex and were unable to oxidize water they were extremely sensitive to photoinhibition. In fact it was necessary to grow the mutants in complete darkness in order to obtain undamaged photosystem II complexes. Based on western blot analyses of D1 protein abundance and size distribution of its proteolytic fragments it was apparent that the H190 mutants were able to assemble an intact PSII complex in the dark, however, the D1 protein was rapidly degraded upon illumination.

In addition, we have also generated a new mutant at residue R269 on the D1 protein. This

residue is suspected to be involved in bicarbonate binding near the Qb binding site and may regulate charge transfer to Qb. This mutant was generated in a collaboration with Dr. Govindjee at the University of Illinois. We have only just begun phenotypic characterization, but it appears as though the mutant does not grow photosynthetically in air. Dr. Ron Hutchison, the postdoc in the lab, has also made significant progress in isolating a psbD clone from *Chlamydomonas* which he is linking up to the streptomycin resistance conferring aadA gene. This construct will be used for positive selection of mutants in the D2 protein. In association with Dr. Jun Minagawa in Dr. Tony Croft's lab, he has constructed an intronless psbA gene linked to the aadA gene for direct positive selection for mutants generated in the D1 protein. Further progress is being made in generating specific D1 and D2 protein mutants. Dr. Hutchison has committed 100% of his efforts towards this project and Dr. Sayre 10% of his efforts. We have also been assisted in our characterization of the mutants by Drs. David Kramer, Klaas van Wiljk and Stenbjorn Styring.

Documents describing the generation and phenotypic description of each of the H190 and H195 mutants have either been submitted for publication or are in preparation for publication. A list of papers submitted or being prepared from our first year is presented below. In addition, our work has been presented at several national and international meetings as listed below.

Publications Submitted or in Preparation:

- 1) Roffey, R.A., Kramer, D., Govindjee and Sayre, R.T. (1993) Characterization of the function of luminal side histidine residues of the chloroplast encoded photosystem II D1 protein. (in preparation for *Biochim. Biophys. Acta*.)
- 2) Kramer, D., Roffey, R.A., Govindjee and Sayre, R.T. (1993) Effects of histidine mutations of the D1 protein on the thermoluminescence A₁ band. (in preparation for *Biochim. Biophys. Acta*.)
- 3) Roffey, R., van Wiljk, K., Sayre, R., Styring, S. (1993) Structural analysis of the environment near the electron donor Z in chloroplast photosystem II complexes. (submitted to *Proc. Nat. Acad. Sciences*).

Papers and Seminars Presented:

- 1) Sayre, R.T. (1992) Structural and Functional Analyses of the photosystem II apparatus of chloroplasts. Biophysics Seminar, Ohio State Univ.
- 2) Sayre, R.T. (1992) Structural and Functional Analyses of the photosystem II Reaction Center Complex of Chloroplasts. Biological Sciences, Purdue University.
- 3) Roffey, R.A. and Sayre, R.T. (1992) Site directed mutagenesis of the photosystem II reaction center. Fifth Int. Conf. on the Cell and Molec. Biol. of *Chlamydomonas*. Asilomar, CA
- 4) Sayre, R.T. (1992) Mutagenesis of the chloroplastic photosystem II apparatus. Univ. Calif. Davis
- 5) Roffey, R.A., Kramer, D., Govindjee and Sayre, R.T. (1992) The effect of luminal side histidine mutations of the D1 protein on photosystem II function. 18th Annual Midwest Photosynthesis Conference, Marshall, IN.
- 6) Roffey, R.A. and Sayre, R.T. (1992) Site directed mutagenesis of the photosystem II reaction center. International Photosynthesis Congress, Nagoya, Japan
- 7) Roffey, R.A. and R.T. Sayre (1993) Altered photosystem II donor side reactions in

mutagenized chloroplasts. First Lawrence Bogorad Symposium, Harvard University

8) Sayre, R.T. (1993) Structural and functional analyses of the photosystem II complex of chloroplasts. Dept. of Biology, University of Waterloo, Ont.

9) van Wijk, K., Roffey, R., Sayre, T. and Styring, S. (1993) Spectroscopic characterization of tyrosine-Z in histidine-190 mutants of the D1 protein of *Chlamydomonas reinhardtii*. European Symposium, Regulation of Gene Expression in *Chlamydomonas*. Amsterdam, The Netherlands.

10) Roffey, R. (1993) Altered electron transfer in photosystem II chloroplast mutants of *Chlamydomonas reinhardtii*. Dept. Plant Biology, Ohio State Univ.

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