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# Difference FT-IR of *Chlamydomonas reinhardtii* photosystem II in the presence and absence of the manganese stabilizing protein

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## Introduction

Photosystem II (PSII) is the multi-subunit protein complex responsible for oxygen production in photosynthetic organisms. PSII uses light energy to catalyze the oxidation of water to molecular oxygen and the reduction of plastoquinone. In recent years, much has been learned about the structure and molecular mechanisms of PSII, however the exact method of water oxidation remains unclear. Our research focuses on using Fourier Transform Infrared spectroscopy (FT-IR) to elucidate the structural changes in PSII; specifically the changes in the manganese stabilizing protein (MSP) associated with illumination and water oxidation.

PSII oxidizes water via a cluster of four manganese atoms, which are located on the luminal side of the thylakoid membrane. The manganese cluster cycles through 5 states (termed $S_n$-states, n = 0-4), accumulating oxidizing equivalents using light energy. Once four oxidizing equivalents have accumulated, a molecule of $O_2$ is released. Conformational changes in PSII are associated with the S-state changes, and much is now known about conformational changes between S-states of intact PSII, in particular the $S_1$-$S_2$ transition. However, no difference FT-IR research has yet been done on PSII isolated from *Chlamydomonas* when extrinsic proteins are removed.

We are interested in the difference in conformational changes in PSII when the 33 kDa PSII extrinsic protein (molecular weight 26 kDa) has been removed. The 33 kDa protein, also known as the manganese stabilizing protein, partially encloses the manganese cluster, and functions in keeping inorganic cofactors necessary for water oxidation ($Ca^{+2}$ and $Cl^-$) in close proximity to the manganese cluster. MSP also acts to stabilize the manganese cluster, presumably through interactions of MSP and the manganese cluster/PSII (Hong et al., 2001). Previous research has shown that aspartic acid and/or glutamic acid residues on Spinach MSP undergo a protonation change during the $S_1$ to $S_2$ transition (Hutchison et al., 1999). Recent research has also indicated the possibility of MSP acting as a channel for transport of water, hydrogen ions, and/or oxygen to or from the active sites of Photosystem II (see Rutherford and Faller, 2001). MSP may also play a structural role in keeping other subunits of PSII (S-LHCII and CP29) in their proper orientations to ensure that excitation energy is transferred properly in PSII (Boekema et al., 2000).

We are studying the role of MSP in the $S_1$ to $S_2$ state transition in water oxidation of PSII harvested from the green algae *Chlamydomonas reinhardtii*. Previous results indicate that the absorbance changes at 200K from $S_1$ to $S_2$ state from *Chlamydomonas* PSII are similar to those from spinach PSII (data not shown). We used light-induced Fourier Transform Infrared (FT-IR) difference spectroscopy to detect light-induced absorbance changes in PSII, both with and without the MSP, following a single flash. This paper will present preliminary results from this study.

**Methods and Materials**

*Chlamydomonas reinhardtii* cells were purified with methods similar to those by Sugiura et al. (1999). *C. reinhardtii* expressing a hexa-histidine tag on the C- terminus of the CP47 protein of the PSII complex were harvested by centrifugation and broken open by nebulization (Glas Col, Terre Haute, IN USA). *C. reinhardtii* contain only one large cup shaped chloroplast per cell; therefore, when the cells were nebulized the chloroplasts are broken open as well. PSII was then solubilized away from the thylakoid membrane using the detergent n-Dodecyl-B-D-Maltopyranoside (lauryl maltoside) (Anatrace, Maumee, Ohio USA). PSII was then purified using column chromatography; a Probond (Invitrogen, Carlesbad, CA USA) nickel affinity column bound the hexa-histadine tag on CP47. After washing off impurities, PSII was eluted using 250mM imidizole, as the imidozole competes with PSII for the nickel binding sites on the column. PSII was then precipitated using polyethylene glycol and washed twice using a 25% glycerol solution followed by 2 washes with 25mM MES. The protein was stored in a sucrose buffer at -20°C before being used for FT-IR spectroscopy. The oxygen production rate of the sample was determined using an Oxygraph (Hansatech, Norfolk, England) oxygen electrode. Chlorophyll concentration was determined as in Patzlaff and Barry (1996).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was done to determine the protein content of the purified PSII sample prior to removal of the extrinsic PSII protein MSP, see Fig. 1. Methods were modified from Neville (1971) as in Piccioni et al. (1982). Neville's buffers, 33.5% Acrylamide/0.9%Bis-Acrylamide were used for gels.

The control sample (MSP not removed) was used without further modification. The extrinsic PSII proteins were removed from the experimental sample with NaCl and urea washes (Hutchison et al., 1998). FT-IR spectroscopy was then done on the different protein complexes (MSP+ and MSP- PSII). The electron acceptors DCBQ and ferricyanide were added in equimolar amounts to the sample to accept electrons from $Q_A$. Concentrated samples were partially dehydrated on a $CaF_2$ window for 20 min under dry $N_2$ gas and loaded into a fourier transform infrared spectrometer (Equinox 55, Bruker Instruments, Billerica, MA USA). The instrument contained a photovoltaic, liquid nitrogen cooled MCT detector with a KBr beamsplitter. The laser light was blocked from the sample using a MIR Bandpass filter (Bruker instruments,3800-500$cm^{-1}$). Samples were illuminated with a Xenon flashlamp (Perkin Elmer, Fremont, CA USA) using a control box (LABPAC, Perkin Elmer). Samples were flashed once every five minutes; scan velocity was set to 160KHz and resolution at 8$cm^{-1}$ with 10 spectra taken every 800 milliseconds. A dark spectrum was taken before each flash and the subsequent scans were ratioed against this dark scan. The resulting spectra were then examined for changes upon illumination and the time following illumination at diagnostic wavenumbers (Fig. 2).

## Results and Discussion

Fig. 1 shows the protein bands present from SDS-PAGE of purified Photosystem II with the various protein subunits of PSII labeled. Protein components of PSII can be observed in the gel: CP47 (47kDa), CP43 (43kDa), D2 (~32kDa), D1 (~32kDa), MSP (~26kDa), and Cyt b-559 (~15kDa). The protein composition is similar to that of Sugiura et al. (1999) for their histidine tagged PSII preparation. The sample was solubilized in a sucrose/MES buffer (at an approximate chlorophyll concentration of 1.2mg/mL and subjected to FT-IR analysis. After the urea wash step, the samples were also resuspended in the same sucrose buffer for FT-IR analysis.

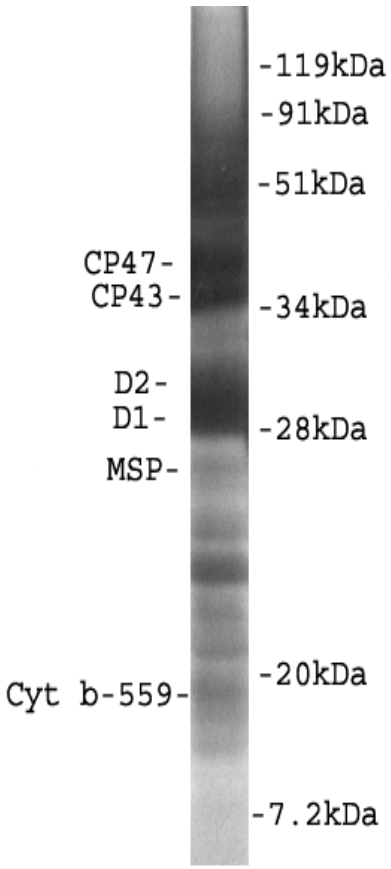


**Figure 1**. SDS-PAGE of purified Photosystem II. PSII protein bands identified on left, molecular weight and position of standards given on right.

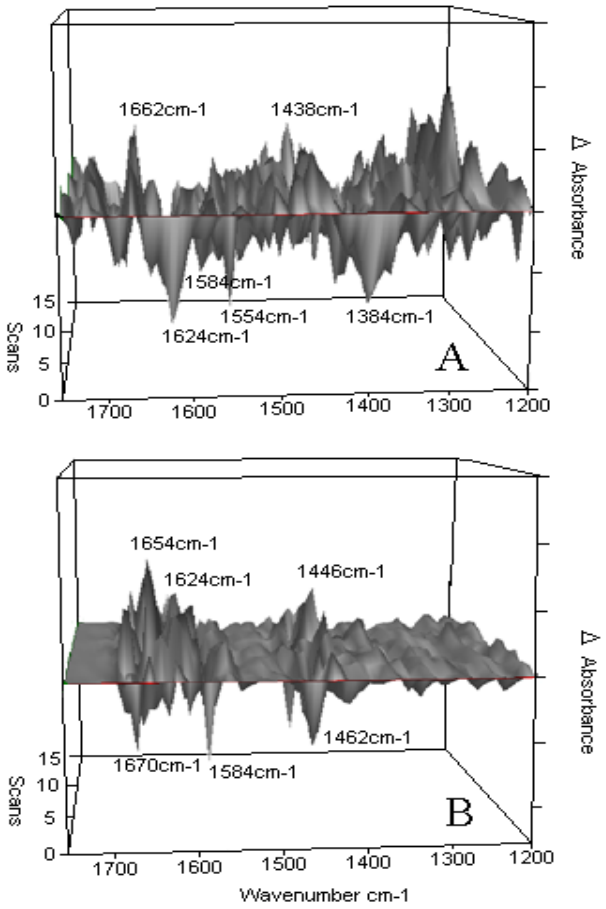


**Figure 2**. FT-IR spectrum of purified PSII for the 12 seconds following illumination. A. MSP containing PSII. B. MSP removed with urea washing. Each spectrum represents an average of 720 scans collected every 800 milliseconds following the flash.

Figure 2 shows a 3D image of 15 spectra taken every 800 milliseconds after a xenon flash. Panel A is the control photosystem II sample and panel B is a photosystem II sample with the MSP removed. We would expect contributions from $Q_A^-$ in these spectra as well as contributions from the $S_2$ state. Both the control and MSP minus samples show a negative absorbance at 1584 $cm^{-1}$ and a positive

absorbance at approximently1660 $cm^{-1}$, indicating possible protein conformational changes in the amide I region. These results suggest that removal of the MSP effects the S1/S2 transition in PSII. This result is consistent with a large body of biochemical data on MSP removal, as well as FT-IR findings on manganese depleted PSII (Chu et al., 2000). The negative feature at 1384 $cm^{-1}$ is also absent from the urea washed sample. Current work in our laboratory will focus on modifying experimental conditions in order to improve signal-to-noise in the rapid scan mode. We also hope to analyze mutants of *Chlamydomonas* MSP using FT-IR. More work is needed to determine the exact molecular workings of the manganese stabilizing protein in the water oxidation reactions of PSII.

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