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Identification of a possible Mn-O-Mn cluster vibrational mode of the S_3 state in the oxygen-evolving complex of photosystem II by low-frequency FTIR difference spectroscopy

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Introduction

Photosynthetic water oxidation occurs in the oxygen-evolving complex (OEC) that is located in the lumenal-side of PSII. The OEC contains a tetranuclear Mn cluster, one Ca ion, possibly several Cl ions, and a redox active tyrosine residue, Y_Z (D_1-Y161) with its associated hydrogen-bonded partner, D_1-H190 (for review, see Britt, 1996, Hoganson & Babcock, 2000; Debus, 2000; Tommos & Babcock, 2000). The water oxidation reaction goes through a cycle of five 'S_n-state' intermediates (n= 0-4, n representing the number of the oxidizing equivalents stored in the OEC). When the S_4 state is reached, O_2 is released and the S_0 state is regenerated. The exact structural arrangement of the OEC and the mechanism of photosynthetic water oxidation is not yet clear. Our goal is to apply light-induced Fourier transform infrared (FTIR) difference spectroscopy to identify the low-frequency metal-ligand and metal-substrate modes from the S-state intermediates of the OEC. These modes are expected to appear in the low-frequency region (1000-200 cm^{-1}) of the IR spectrum (Chu *et al*., 2001). Identification of these low-frequency vibration modes from the S state intermediates of the OEC would provide direct structural evidence for the mechanism of photosynthetic water oxidation. However, there are several technical obstacles that complicate the low-frequency IR measurement, including strong water absorbance, limitations in optical materials, and detector sensitivity. These obstacles have limited previous FTIR spectroscopic studies of the OEC to higher frequencies ($\geq$ 1000 cm^{-1}). Recently we have overcome these technical difficulties and have reported low-frequency S_2/S_1 spectra down to 350 cm^{-1} (Chu *et al*., 1999, 2000a,b; 2001). By exchanging PSII samples with buffered ^{18}O water and by exchanging Ca^{2+} with Sr^{2+} and $^{44}Ca^{2+}$, we were able to assign an S_2 mode at 606 cm^{-1} in the S_2/S_1 spectrum to a Mn-O-Mn cluster vibrational mode of the OEC (Chu *et al*., 2000a). This Mn-O-Mn cluster structure may contain additional bridge(s) that could correspond to another oxo, carboxylato(s), or atoms derived from an amino acid side chain. Our results also indicate that the bridged oxygen atom in this Mn-O-Mn cluster is exchangeable and accessible by water (Chu *et al*., 2000a). In this communication, we present our preliminary results on structural changes of the OEC during S_2-to-S_3 state transition.

Materials and Methods

Spinach PSII OTG (Octyl-β-D-thioglucopyranoside) reaction center cores (RCCs), retaining all the three extrinsic polypeptides, were prepared as described in

[1] *This contribution is dedicated to the memory of Gerald T. Babcock.*

Mishra & Ghanotakis, 1994. FTIR experiments were performed on a Bomen 102 spectrometer with a liquid-He cooled Si bolometer. Samples were cooled to 250K by using a home-built liquid nitrogen cryostat and the sample temperature was controlled to ±0.1°C with a temperature controller (LakeShore 321). Samples were illuminated by one or two single flashes from a frequency-doubled Nd:YAG laser (Quanta-Ray DCR-11) (532nm, ~7ns, ~30 mJ/pulse cm^2). For each sample, three single beam spectra (~3 minutes duration, 150 scans, 4 cm^{-1} resolution for each) were recorded (one before the flashes, one after the first flash, and one after the second flash, respectively). Single-flash (S_2/S_1) spectra were generated by ratioing the dark single beam spectrum with the first-flash spectrum. Two-flashes (S_3/S_1) spectra were generated by ratioing the first-flash single beam spectrum with the second-flash spectrum. Multiple spectra (3-6) were averaged to improve the S/N.

Results and Discussion

Figure 1 shows the low-frequency FTIR difference spectra obtained from hydrated, O_2-evolving OTG RCCs induced by one flash (top spectrum, solid line) and by two flashes (top spectrum, dotted line) at 250K in the presence of ferricyanide. The S_3/S_2 spectrum (bottom spectrum) was generated by subtracting the normalized one flash spectrum from the two flash spectrum. We found that several low-frequency IR modes in the S_3/S_2 spectrum are associated with bond rearrangements induced by the second laser flash, *e.g.*, the positive bands at 621, 590 and 539 cm^{-1} and negative bands at 604 and 566 cm^{-1}. The positive mode at ~590 cm^{-1} corresponds

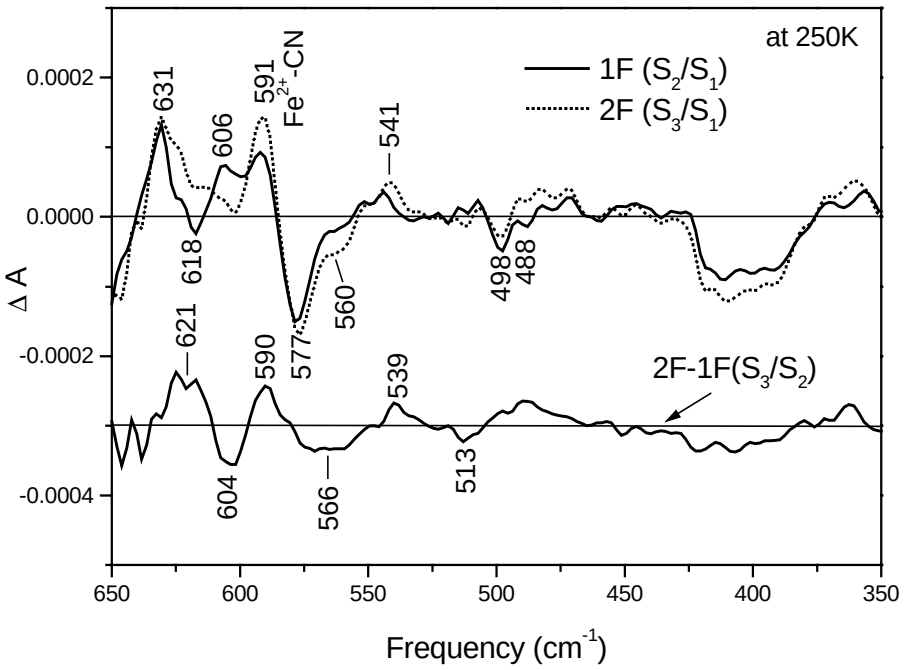

Fig. 1. Low-frequency FTIR difference spectra obtained from hydrated, O_2-evolving PSII RCCs induced by one flash (top spectrum, dotted line) and by two flashes (top spectrum, dashed line) at 250K in the presence of ferricyanide. The S_3/S_2 spectrum (bottom spectrum) is generated by subtracting the normalized one-flash spectrum from the two-flash spectrum.

to the Fe-C stretch mode of ferrocyanide and is present in both S_2/S_1 and S_3/S_2 spectra. The appearance of the ferrocyanide band in the difference spectra indicates that after each flash the ferricyanide become reduced by accepting an electron from Q_A^-. In our previous work, we assigned the S_2 mode at 606 cm^{-1} in the S_2/S_1 spectrum to a Mn-O-Mn cluster vibration of the OEC (see Figure 1 and Chu *et al*. 2000a). The presence of the S_2 mode (negative band) at 604 cm^{-1} in the S_3/S_2 spectrum suggests that the same Mn-O-Mn cluster might undergo structural changes during S_2-to-S_3 transition as well. In addition, judging from its intensity and frequency, the S_3 mode at ~621 cm^{-1} in the S_3/S_2 spectrum would be a good candidate for the corresponding Mn-O-Mn cluster mode of the S_3 state.

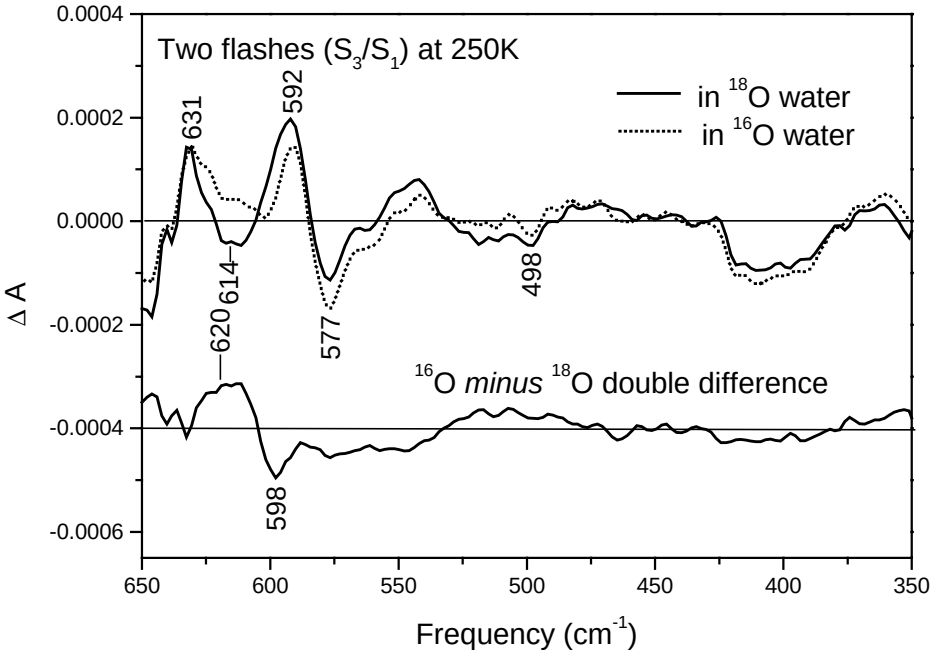

Fig. 2. Low-frequency FTIR difference spectra of hydrated spinach PSII RCCs in buffered ^{16}O water (solid line) or ^{18}O water (dotted line) induced by the two flashes at 250K. The double difference (^{16}O minus ^{18}O) spectrum is generated by subtracting the ^{18}O spectrum from the ^{16}O spectrum.

Figure 2 shows FTIR difference spectra of hydrated spinach PSII RCCs in buffered ^{16}O water (solid line) or ^{18}O water (dotted line) induced by two flashes at 250K, in the presence of ferricyanide. We found that the positive band at ~621 cm^{-1} in the two-flash spectrum (containing most of S_3/S_1) is clearly down shifted and overlapping with the ferrocyanide band at ~590 cm^{-1} upon ^{18}O water exchange (Figure 2, dotted line). The 620/598 cm^{-1} set of difference peaks apparent in the double-difference spectrum (Figure 2, bottom spectrum) provides convincing evidence that we have observed an S_3 mode that involves an exchangeable oxygen in its normal coordinate. Our preliminary results from S_3/S_2 spectra in ^{18}O water show that the S_2 mode at 604 cm^{-1} in the S_3/S_2 spectrum is also sensitive to ^{18}O water exchange.

The above results may be interpreted in at least one of three ways. A first possibility is that the S_3 mode at ~621 cm^{-1} has a different Mn-O-Mn cluster origin

than the S_2 state Mn-O-Mn cluster mode at 606 cm^{-1} in the S_2/S_1 spectrum. In addition, our results show that the ^{18}O shift (~23 cm^{-1}) of this S_3 mode at ~621 cm^{-1} is about two times larger than that (~10 cm^{-1}) of the Mn-O-Mn cluster mode of the S_2 state at 606 cm^{-1} (see Fig 2 and Chu *et al*. 2000a). Different origins would explain why these two vibrational modes have different shifts upon ^{18}O water exchange. Another possibility is that the S_3 mode at ~621 cm^{-1} and the S_2 mode at 604 cm^{-1} originate from the same Mn-O-Mn moiety that gives rise to the S_2 mode at 606 cm^{-1}. Based on the isotopic labeling results described (see Fig 2 and Chu *et al*. 2000a), if the S_3 mode at ~621 cm^{-1} and the S_2 mode at 604 cm^{-1} in S_3/S_2 spectrum originated from the same Mn-O-Mn cluster of the OEC, which gives rise to the S_2 mode at 606 cm^{-1} in the S_2/S_1 spectrum, this would suggests that there is a significant change in the structure of this Mn-O-Mn cluster itself and/or its immediate protein surrounding during the S_2-to-S_3 state transition. In the previous study (Chu *et al.*, 2000a), we proposed that if the structure of this Mn-O-Mn cluster is a Mn_2 di-μ-oxo core, then the small ^{18}O shift (~10 cm^{-1}) of the S_2 mode at 606 cm^{-1} suggested that only one of the bridged oxygen atoms in the Mn-O-Mn cluster is accessible and exchangeable by water in the S_2 state. The ^{18}O-induced shift (~23 cm^{-1}) of the S_3 mode at ~621 cm^{-1} suggests that both bridged oxygen atoms in the Mn-O-Mn cluster become accessible and exchangeable by water in the S_3 state. Alternatively, a structural change on this Mn-O-Mn cluster during S_2-to-S_3 transition might account for the ^{18}O-induced shift as has been proposed based on recent EXAFS studies (Liang *et. al*. 2000, Dau *et. al*. 2001). Currently we are in the process of testing the above possibilities by combining isotopic labeling, model compound studies and normal mode analysis with this low-frequency FTIR approach.

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