Identification of a possible Mn-O-Mn cluster vibrational mode of the S₃ 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 tetracovalent Mn cluster, one Ca ion, possibly several Cl ions, and a redox active tyrosine residue, Y₁₆₁(D₁-Y₁₆₁) with its associated hydrogen-bonded partner, D₁-H₁₉₀ (for review, see Britt, 1996, Hoganson & Babcock, 2000; Debus, 2000; Tommos & Babcock, 2000). The water oxidation reaction goes through a cycle of five ‘Sₙ-state’ intermediates (n= 0-4, n representing the number of the oxidizing equivalents stored in the OEC). When the S₄ state is reached, O₂ is released and the S₃ 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 (≥ 1000 cm⁻¹). Recently we have overcome these technical difficulties and have reported low-frequency S₂/S₁ spectra down to 350 cm⁻¹ (Chu et al., 1999, 2000a,b; 2001). By exchanging PSII samples with buffered ¹⁸O water and by exchanging Ca²⁺ with Sr²⁺ and ⁴⁴Ca²⁺, we were able to assign an S₂ mode at 606 cm⁻¹ in the S₂/S₁ 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 S₂-to-S₃ state transition.

Materials and Methods

Spinach PSII OTG (Octyl-β-D-thioglycoside) 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 cm²). 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 (S₂/S₁) spectra were generated by ratioing the dark single beam spectrum with the first-flash spectrum. Two-flashes (S₃/S₁) 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, O₂-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 S₃/S₂ 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 S₃/S₂ 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

![Graph](image_url)

**Fig. 1.** Low-frequency FTIR difference spectra obtained from hydrated, O₂-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 S₃/S₂ 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 S2/S1 and S3/S2 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 QA. In our previous work, we assigned the S2 mode at 606 cm\(^{-1}\) in the S2/S1 spectrum to a Mn-O-Mn cluster vibration of the OEC (see Figure 1 and Chu et al. 2000a). The presence of the S2 mode (negative band) at 604 cm\(^{-1}\) in the S3/S2 spectrum suggests that the same Mn-O-Mn cluster might undergo structural changes during S2-to-S3 transition as well. In addition, judging from its intensity and frequency, the S3 mode at \(\sim 621\) cm\(^{-1}\) in the S3/S2 spectrum would be a good candidate for the corresponding Mn-O-Mn cluster mode of the S3 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 \(\sim 621\) cm\(^{-1}\) in the two-flash spectrum (containing most of S3/S1) is clearly down shifted and overlapping with the ferrocyanide band at \(\sim 590\) 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 S3 mode that involves an exchangeable oxygen in its normal coordinate. Our preliminary results from S3/S2 spectra in \(^{18}\)O water show that the S2 mode at 604 cm\(^{-1}\) in the S3/S2 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 S3 mode at \(\sim 621\) 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 ($\sim$23 cm$^{-1}$) of this $S_3$ mode at $\sim$621 cm$^{-1}$ is about two times larger than that ($\sim$10 cm$^{-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 $\sim$621 cm$^{-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 $\sim$621 cm$^{-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-$\mu$-oxo core, then the small $^{18}$O shift ($\sim$10 cm$^{-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 ($\sim$23 cm$^{-1}$) of the $S_3$ mode at $\sim$621 cm$^{-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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References