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# Properties of $Cl^-$ Binding Site in Oxygen-Evolving Complex of Photosystem II Studied by FTIR Spectroscopy

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Role of $Cl^-$ in photosynthetic oxygen-evolving complex was studied by light-induced Fourier transform infrared (FTIR) spectroscopy. $Cl^-$ depletion resulted in the suppression of amide I and amide II IR modes upon $S_1$ to $S_2$ transition. $Br^-$, $I^-$, and $NO_3^-$ substituted FTIR difference spectra were very similar to that in $Cl^-$ reconstitution. $F^-$ and $CH_3COO^-$ substituted spectra were largely distorted. We succeeded in detecting the structural change of $NO_3^-$ in the $Cl^-$ site upon the $S_1$ to $S_2$ transition from $^{14}NO_3^-/^{15}NO_3^-$ difference spectrum.

**Key words:** chloride, FTIR, Mn-cluster, oxygen-evolving complex, photosystem II

## INTRODUCTION

Photosynthetic oxygen evolution is carried out by an oxygen-evolving complex (OEC) residing on the donor side of photosystem (PS) II. The OEC involves a tetranuclear Mn-cluster that provides a catalytic site for water oxidation. The reaction comprises of five intermediate states labeled $S_i$ (i=0–4), where $S_n$ proceeds to $S_{n+1}$ by absorbing a photon (n=1 in the dark). $Cl^-$ is an essential inorganic cofactor to OEC function, and its depletion impairs the water oxidation capability. $Cl^-$ can be functionally replaced by another monovalent anion such as $Br^-$, $I^-$ and $NO_3^-$ but not by $F^-$ and $CH_3COO^-$. Although it has been believed that $Cl^-$ is closely associated with OEC, precise role and location of $Cl^-$ in OEC are largely unknown.

Light-induced FTIR spectroscopy is a powerful method to investigate the molecular structure and reaction process in OEC. In this work, we report effects of $Cl^-$ depletion and anion substitution on the mid-frequency $S_2/S_1$ FTIR difference spectrum. On the basis of the obtained results, the properties of the $Cl^-$ site and the role of $Cl^-$ in OEC are discussed.

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## MATERIAL AND METHOD

BBY-type PSII membranes capable of oxygen evolution were prepared from spinach, and PS II core complexes in mutant lacking $Tyr_D$ (D2-Tyr160Phe) were prepared from *Chlamydomonas reinhardtii*. For $Cl^-$ depletion, the PS II samples were washed with 2M NaCl buffer (pH 6.5), and resuspended seven times in $Cl^-$ free buffer containing 400 mM sucrose, 20 mM MES-NaOH (pH 6.5) with one-tenth volume of a medium containing 100 mM $Ca(OH)_2$ and 300 mM MES (pH 6.4). $S_2/S_1$ FTIR difference spectrum, which shows the structural changes due to the oxidation of Mn-cluster in dark adapted OEC, was obtained by subtracting $Q_A^-/Q_A$ difference spectrum from $S_2Q_A^-/S_1Q_A$ difference spectrum at 250 K with CW illumination in the presence of DCMU ($Q_A$ is a primary electron acceptor).

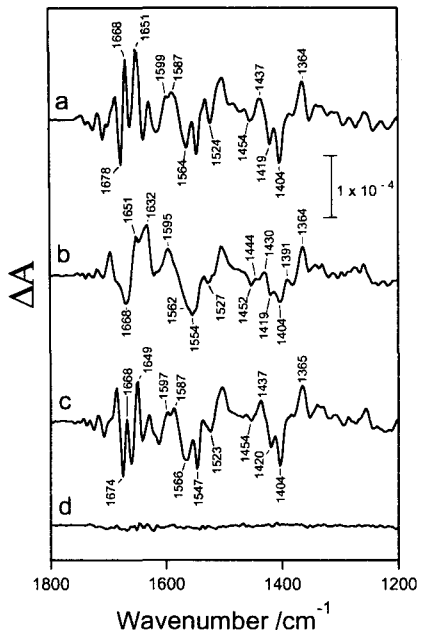


Figure 1: Light-induced $S_2/S_1$ FTIR difference spectra of spinach PS II membranes that are (a) untreated, (b) $Cl^-$ depleted and (c) $Cl^-$ reconstituted with 40 mM NaCl. (d) Noise level.

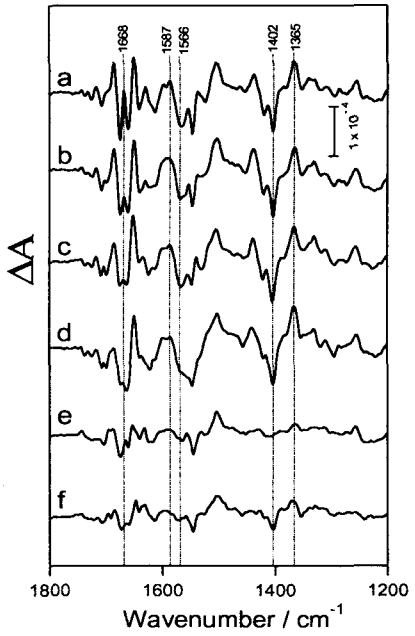


Figure 2: Light-induced $S_2/S_1$ FTIR difference spectra of $Cl^-$-depleted spinach PS II membranes that are reconstituted with (a) $Cl^-$, (b) $Br^-$, (c) $I^-$ (d) $NO_3^-$, (e) $F^-$ and (f) $CH_3COO^-$. For reconstitution, sample membranes were supplemented with 40 mM Na-salt of each anion.

## RESULTS AND DISCUSSION

Figure 1 shows the effects of $Cl^-$ depletion on the $S_2/S_1$ FTIR difference spectrum. By $Cl^-$ depletion, the amide I (1690–1630 $cm^{-1}$) and II (1590–1515 $cm^{-1}$) IR bands due to the structural changes of protein backbone were largely suppressed or disappeared, while the bands at 1587(+)/1564(−) $cm^{-1}$ for asymmetric and at 1364(+)/1404(−) $cm^{-1}$ for symmetric stretching modes of the putative carboxylate ligands for the Mn-cluster [1] still remained considerably. This result indicates that $Cl^-$ is required for the structural changes of the protein backbone upon $S_1$ to $S_2$ transition, and the suppression of the change may be ascribed to the inhibition of normal S state turnover beyond the $S_2$ state in $Cl^-$ depletion.

Figure 2 shows the effects of monovalent anion substitution on the $S_2/S_1$ FTIR difference spectrum. The overall features of $Br^-$, $I^-$, and $NO_3^-$ substituted spectra were very similar to the untreated, and $Cl^-$ reconstituted spectra, being consistent with their capability in supporting oxygen-evolution. However the amide I band at 1668(+) $cm^{-1}$ is barely recovered by $I^-$ and $NO_3^-$ substitution, suggesting that this band is not essential for the normal function of OEC. The spectral features of $F^-$ and $CH_3COO^-$ substitutions remarkably differ from those of surrogate anions $Cl^-$, $Br^-$, $I^-$, and $NO_3^-$. The marked suppression of the $S_2/S_1$ band formations in the $F^-$ and $CH_3COO^-$ substituted spectra might be ascribed to an electron donation from some redox component in competition with the Mn-cluster. Since $Tyr_D$ is a possible candidate for this alternative component, we measured $S_2/S_1$ FTIR difference spectra in the core complexes from $Tyr_D$ less mutant of *C. reinhardtii*, as showing in Figure 3. The normal difference spectrum was induced in presence of $Cl^-$ but the formation of the bands was largely suppressed by $F^-$ and $CH_3COO^-$ substitutions. This indicates that electron donation from $Tyr_D$ is not responsible for the suppression. Therefore, it

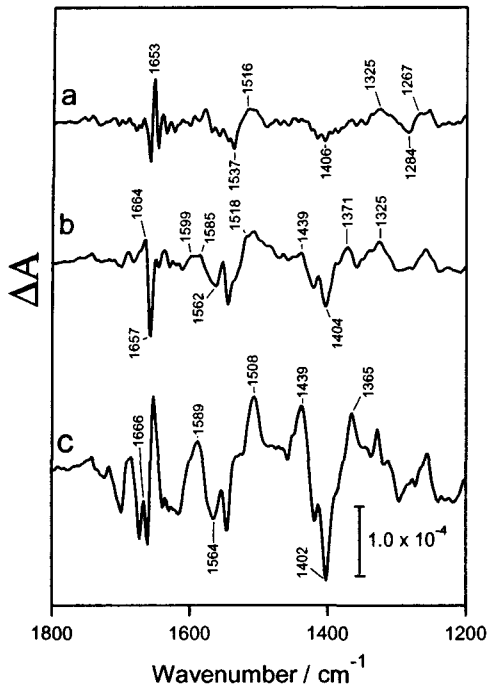


Figure 3: Light-induced $S_2/S_1$ FTIR difference spectra of (a) $F^-$, (b) $CH_3COO^-$ substituted and (c) $Cl^-$ reconstituted $Tyr_D$ less PS II core complexes of *C. reinhardtii*.

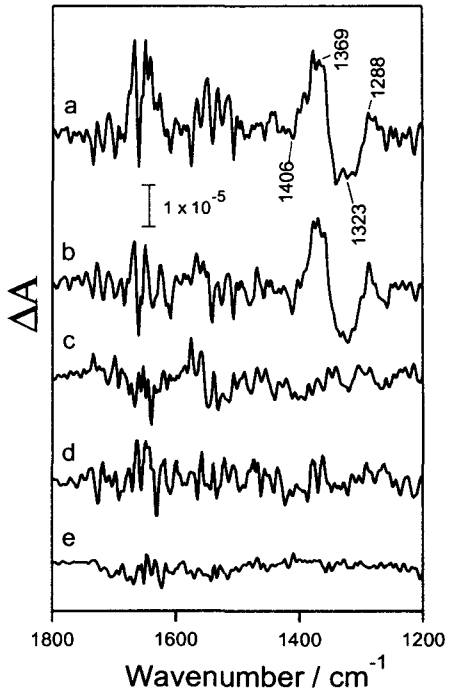


Figure 4: $^{14}NO_3^-/^{15}NO_3^-$ FTIR difference spectra for (a) light-induced $S_2/S_1$ FTIR difference spectrum, (b, d) light-induced $S_2Q_A^-/S_1Q_A$ FTIR difference spectrum and (c) light-induced $Q_A^-/Q_A$ difference spectrum. For spectrum (d), 20 mM NaCl was further included in the sample suspension. (e) Noise level.

may imply that the binding of $F^-$ or $CH_3COO^-$ prevents structural changes of protein matrices proximal to the Mn-cluster upon its oxidation to the $S_2$ state.

As shown in Figure 2, $NO_3^-$ can be functionally substituted for $Cl^-$, indicating that $NO_3^-$ is bound to the $Cl^-$ binding site. Since vibrational modes of $NO_3^-$ are very sensitive to its binding form, $NO_3^-$ can be used as a potent probe to elucidate the binding properties of $Cl^-$ to its site. Figure 4 shows $^{14}NO_3^-/^{15}NO_3^-$ FTIR difference spectra. The $^{14}NO_3^-/^{15}NO_3^-$ ($S_2/S_1$ and $S_2Q_A^-/S_1Q_A$) difference spectrum clearly showed the isotopic bands, which appear a prominent positive band at ~1370 $cm^{-1}$ and a negative band at ~1323 $cm^{-1}$ with minor positive and negative bands at ~1288 and ~1405 $cm^{-1}$. On the basis of these band position, the bands are ascribed to asymmetric NO stretching modes of an ionic $NO_3^-$ but not metal-binding $NO_3^-$. No isotopic band was observed in the $Q_A^-/Q_A$ difference spectrum, as well as the $S_2Q_A^-/S_1Q_A$ difference spectrum by further supplementation of $Cl^-$, indicating that the isotopic bands arise from structural changes of $NO_3^-$ which is bound to the $Cl^-$ binding site. These results demonstrate that the $Cl^-$ binding site is structurally coupled with the Mn-cluster, but $Cl^-$ ($NO_3^-$) is not direct ligand for the Mn-cluster.

## REFERENCES

1. Kimura, Y. and T.-a. Ono (2001) Chelator-induced disappearance of carboxylate stretching vibrational modes in $S_2/S_1$ FTIR spectrum in oxygen-evolving complex of photosystem II. *Biochemistry* 40, 14061-14068.