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Review

Fourier transform infrared difference and time-resolved infrared detection of the electron and proton transfer dynamics in photosynthetic water oxidation☆,☆☆

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ABSTRACT

Photosynthetic water oxidation, which provides the electrons necessary for CO_2 reduction and releases O_2 and protons, is performed at the Mn_4CaO_5 cluster in photosystem II (PSII). In this review, studies that assessed the mechanism of water oxidation using infrared spectroscopy are summarized focusing on electron and proton transfer dynamics. Structural changes in proteins and water molecules between intermediates known as S_i states ($i = 0$–3) were detected using flash-induced Fourier transform infrared (FTIR) difference spectroscopy. Electron flow in PSII and proton release from substrate water were monitored using the infrared changes in ferricyanide as an exogenous electron acceptor and Mes buffer as a proton acceptor. Time-resolved infrared (TRIR) spectroscopy provided information on the dynamics of proton-coupled electron transfer during the S-state transitions. In particular, a drastic proton movement during the lag phase (~200 μs) before electron transfer in the $S_3 \rightarrow S_0$ transition was detected directly by monitoring the infrared absorption of a polarizable proton in a hydrogen bond network. Furthermore, the proton release pathways in the PSII proteins were analyzed by FTIR difference measurements in combination with site-directed mutagenesis, isotopic substitutions, and quantum chemical calculations. Therefore, infrared spectroscopy is a powerful tool for understanding the molecular mechanism of photosynthetic water oxidation. This article is part of a Special Issue entitled: Vibrational Spectroscopies in Molecular Bioenergetics.

1. Introduction

In the oxygenic photosynthesis performed by plants and cyanobacteria, light energy is converted into chemical energy in the form of sugars using only water and CO_2 as the chemical resources. Water serves as the ultimate electron donor for the reduction of CO_2, and is split into O_2 and protons when oxidized. The protons are released into the thylakoid lumen to produce a proton gradient across the thylakoid membranes, which drives ATP synthesis to serve as an energy source for CO_2 fixation. In contrast, O_2 is liberated into the air, creating an oxygenic atmosphere (21% O_2) that is used for respiration. O_2 is also converted into ozone, which protects life from harmful UV radiation. Therefore, oxygenic photosynthesis is a biological process essential for sustaining the global environment and life on earth as a source of both energy and O_2.

Photosynthetic water oxidation is performed in the photosystem II (PSII) protein complexes [1–8]. In PSII, electron transfer starts from the excited singlet state of the reaction center chlorophylls (the coupled excited state of monomeric chlorophyll Chl_{D1} and chlorophyll dimer P680), which ejects an electron to the pheophytin electron acceptor Pheo, and leads to the formation of a $P680^+Pheo^-$ charged pair [9,10] (Fig. 1A). On the electron acceptor side, an electron is transferred from $Pheo^-$ to the primary quinone electron acceptor Q_A and then to the secondary acceptor Q_B [11]. Upon accepting two electrons, Q_B is converted into a plastoquinole molecule by the uptake of two protons, and is then released into the thylakoid membranes. On the electron donor side, $P680^+$ oxidizes the redox-active tyrosine Y_Z (D1-Y161) followed by the water oxidizing center (WOC), which is the catalytic site of water oxidation [1–8]. Therefore, electrons extracted from water are transferred finally to plastoquinol, via light-driven reactions in PSII, which transports electrons to the Cytb_6/f complex in thylakoid membranes.

X-ray crystallographic structures of PSII complexes [12–14] show that the WOC consists of the Mn_4CaO_5 cluster, two Cl^- ions located ~7 Å away from the closest Mn ions, and surrounding amino acid residues including ligands for the Mn and Ca ions [D1-Asp170, D1-Glu189, D1-Asp333, D1-Asp342, D1-Ala344 (C-terminus), CP43-Glu354, and D1-His332] (Fig. 2). A recent high-resolution (1.9 Å) X-ray structure [14] further resolved the water ligands: W1 and W2

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☆☆ This article is dedicated to the memory of Warwick Hillier (Oct. 18, 1967–Jan. 10, 2014), who made significant contributions to the water oxidation researches using FTIR spectroscopy and mass spectrometry.

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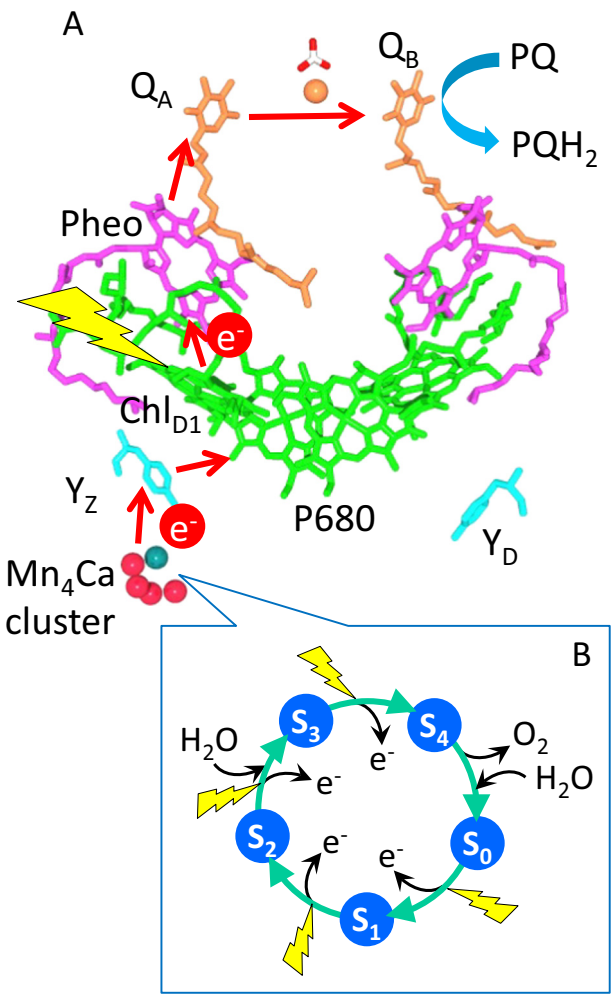

Fig. 1. (A) Arrangements of redox cofactors in photosystem II and the electron transfer pathway. (B) S-state cycle of water oxidation at the Mn_4CaO_5 cluster.

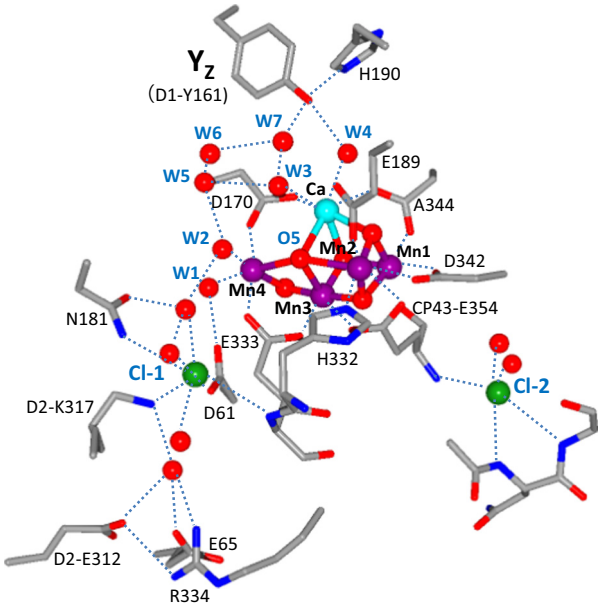

Fig. 2. Structure of WOC deduced from the X-ray crystal structure of photosystem II at 1.9 Å resolution (PDB ID: 3ARC [14]). Amino acid residues in which the subunit name is not specified in the labels are all on the D1 subunit. The numbering of the atoms in the Mn_4CaO_5 cluster, Cl^- ions, and water molecules follows that of Umena et al. [14] and Kawakami et al. [128].

were ligated to Mn4, and W3 and W4 were ligated to Ca (the numbering follows Umena et al. [14]). In addition to these four water molecules, the oxygen atom O5, which is located nearly equidistant from Ca, Mn4, Mn3 and Mn1 in the Mn_4CaO_5 cluster, is a possible candidate substrate [14–16]. Hydrogen bond networks involving these water ligands are formed around the Mn_4CaO_5 cluster. In particular, a water cluster consisting of W3, W4, W5, W6 and W7 is present between the Mn_4CaO_5 cluster and Y_Z, whereas a hydrogen bond network involving D1-Asp61, D2-Lys-317, D1-Glu65, D2-Glu312, D1-Arg334 and several water molecules is formed around one of the Cl^- ions (Cl-1) (Fig. 2). Such hydrogen bond networks likely play a crucial role in proton release processes during water oxidation.

At the WOC, two water molecules are oxidized into one O_2 molecule and four protons through the cycle of five intermediates designated S_i states ($i = 0$–4); a larger i value implies a higher oxidation state of the Mn_4CaO_5 cluster (Fig. 1B) [1–8]. The S_1 state is most stable in the dark, and the S_i state ($i = 0$–3) advances to the next S_{i+1} state upon the extraction of one electron. The S_4 state is a transient intermediate that immediately relaxes to the S_0 state by releasing O_2. However, there are several unanswered questions in the water oxidation mechanism. For example, it remains unclear how the electron and proton transfer reactions are coupled with each other in individual S-state transitions. It is also unknown in which order the electron and protons are released from the WOC. Similarly, the pathway in the PSII proteins for the release of each proton and the energetic requirement that drives the reaction are yet to be elucidated. Answering these questions is essential to achieve a full understanding of the mechanism of photosynthetic water oxidation.

Infrared (IR) spectroscopy, particularly light-induced Fourier transform infrared (FTIR) difference spectroscopy [17–19], has been used extensively to study the mechanism of photosynthetic water oxidation [20–28]. Flash-induced FTIR difference spectra have been measured upon individual transitions in the S-state cycle ($S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, $S_3 \rightarrow S_0$ and $S_0 \rightarrow S_1$) [29,30], and data regarding the structures and reactions of the proteins and water molecules during water oxidation have been obtained. Time-resolved infrared (TRIR) spectroscopy was also used to monitor the movements of electrons and protons *during* the S-state transitions of WOC reaction [31]. In this review, I summarize the applications of light-induced FTIR difference and TRIR spectroscopies for investigating the molecular mechanism of photosynthetic water oxidation, focusing on the coupling of electron and proton transfer reactions. For other topics, such as ligand structure, water reactions, lower-frequency Mn cluster vibrations, and the effects of extrinsic proteins, I refer readers to previous reviews [20–28].

2. Flash-induced FTIR difference spectra of the S-state transitions

Fig. 3 shows flash-induced FTIR difference spectra of the S-state transitions measured using PSII core complexes from the cyanobacterium *Thermosynechococcus elongatus* in the presence of ferricyanide as an exogenous electron acceptor [29,32,33]. Because electrons are abstracted by ferricyanide to the outside of proteins, IR changes coupled to the WOC reactions are obtained without interference from acceptor side signals, except for the CN stretching vibrations of ferricyanide/ferrocyanide at 2116/2038 cm^{-1}. Difference spectra upon the 1st, 2nd, 3rd and 4th flashes virtually represent structural changes in the $S_1 \rightarrow S_2$, $S_2 \rightarrow S_3$, $S_3 \rightarrow S_0$ and $S_0 \rightarrow S_1$ transitions, respectively, although minor contributions of other transitions are mixed into the spectra at the later flashes due to ~10% miss probabilities (see Section 3).

Numerous signals are found in the mid-frequency region of 1800–1000 cm^{-1}, where protein bands mainly appear [34,35], which is indicative of drastic protein movements during water oxidation. The presence of several prominent bands in the amide I (the CO stretch of backbone amide) region at 1700–1600 cm^{-1} indicates that the

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