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*Taking Snapshots of Photosynthetic Water Oxidation with an X-ray Laser*

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Light-induced oxidation of water in the photosystem II (PS II) membrane protein complex is catalyzed by a Mn4CaO5 cluster and this reaction is responsible for most of the dioxygen in the atmosphere. We have developed and utilized new methodologies of X-ray spectroscopy and crystallography using X-ray free electron laser (XFEL) sources, to investigate the structural and electronic structural changes of the Mn4CaO5 cluster and to understand the mechanism by which H2O is oxidized to O2 in the PS II protein complex during the catalytic reaction. We developed the method of simultaneous X-ray diffraction (XRD) and X-ray emission spectroscopy (XES) using XFELs, and using this method we collected time-resolved crystallography and spectroscopy data of PS II at room temperature. We obtained the structures of cyanobacterial PS II in the dark and various intermediate states (called Si-states, where i=0-4) at better than 2.5 Å resolution, using the femtosecond pulses of the XFEL at Stanford, the LCLS, and demonstrated that we can proceed through the entire intermediate S-state cycle, including time-points between the S3 and S0 states, where the O-O bond formation and O2 evolution is proposed to occur. The S-state intermediates of PS II, in the S2, S3, S0 and the time-points between the S3 and S0 states, were generated, in situ, by multiple visible laser excitations. Distinct differences in the overall structure compared to the reported cryogenic temperature structures are observed, which include higher side-chain mobility with multiple conformers, expansion of the dimer in the membrane plane with changes in the helix orientations, and longer cofactor-cofactor distances. On the other hand, in the dark S1 state, the Mn4CaO5 cluster is similar to the structure at cryogenic temperature. Major structural changes are not observed either in the peptide backbone or the Mn4CaO5 cluster between the dark and illuminated states, precluding mechanisms that require large changes in the intermediate state. Isomorphous difference maps between the various S-states and the time-points between the S3 and S0 states have been obtained which show changes at the Mn4CaO5 catalytic center and vicinity and these will be discussed in the context of the mechanism of the water oxidation reaction. The Kβ X-ray emission spectra, simultaneously collected with XRD, showed that the Mn cluster was undamaged and advanced through the S-states as characterized by the changes in the X-ray emission spectra. The emission spectra from time-points between the S3 to S0 transition demonstrate that the kinetics during the O-O bond formation step and oxidation/reduction of the Mn4CaO5 cluster are complex. We also developed a method to collect Mn L-edge spectra of PS II at room temperature using XFELs, based on a new high transmission zone-plate spectrometer that can discriminate between the O background and the Mn signal, to study the detailed electronic structural changes of the Mn4CaO5 cluster during the catalysis. The different classes of suggested water oxidation mechanisms will be discussed in light of our results from XFEL based room temperature X-ray spectroscopy and X-ray crystallography of PS II in the dark and the intermediate states.

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