	Experiment title: The Mn oxidizing S2-S3 transition of the catalytic cycle of photosynthetic O ₂ evolution: Characterization of an intermediate by time-resolved XAS	Experiment number: SC2478
Beamline: ID26	Date of experiment: from: 10.06.2008 to: 17.06.2008	Date of report: 04.09.2008
Shifts: 21	Local contact(s): Dr. Tsu-Chien Weng	<i>Received at ESRF:</i>
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Report:

Understanding the mechanism of photosynthetic dioxygen production is a contemporary challenge in the strive to replace fossil fuels by renewable sources. Recent time-resolved X-ray absorption spectroscopy experiments have enabled us to detect a new intermediate in the crucial oxygen evolving step in the catalytic reaction cycle of water oxidation at the manganese complex of photosystem II (PSII) [1-5]. Time-resolved X-ray experiments under elevated O₂ partial pressure revealed the absence of inhibition of O₂ formation by product backpressure, but suggested additional Mn oxidation [6]. In the present investigation, we attempted to kinetically label and stabilize those intermediates in the catalytic cycle which are related to protolytic reactions. Therefore, time resolved XAS experiments were carried out on PSII preparations where H₂O was exchanged against D₂O. In particular, we were interested in the S2-S3 transition, for which the oxidation of Mn is likely, but still disputed, and previous experiments in our laboratory provided preliminary evidence for the existence of an intermediate. Time-resolved X-ray transients with unprecedented quality were obtained. The data strongly favours Mn oxidation on S2-S3 and, even more important, supports the existence of a short-lived intermediate. Intermediate formation likely is coupled to a proton transfer step.

Experimental:

Partially dehydrated PSII membrane fragment samples for time-resolved XAS were prepared as previously. Here, the whole preparation procedure was carried out in D₂O. A total of ~20000 PSII samples was used. Timescans of the X-ray fluorescence at 10 excitation energies in the region of the Mn K-edge were recorded, during laser-flash excitation of the PSII samples in the X-ray beam. Up to 2000 timescan traces were recorded at a single excitation energy and averaged. In addition, Mn K-edge spectra were recorded on PSII samples where the individual S-states of the Mn complex were populated by the application of 0-6 Laser flashes prior to the rapid scan (1 s duration).

Results:

(1) Figure 1 shows timescan traces of the X-ray fluorescence at an excitation energy of 6552.5 eV of PSII samples in D₂O on the S1-S2 (black), S2-S3 (red), oxygen-evolving S3-S0 (green), and S0-S1 (blue) transitions. Time-resolution is 10 μ s per data point. The traces represent the average of ~3000 transients each measured on a fresh PSII sample and were corrected for imperfect advancement of the PSII population through the S-state cycle in a series of laser flashes. Due to improvements of our experimental setup, the data show an unprecedented signal-to-noise ratio. Analysis of the transients by curve fitting allows for the detection of kinetic phases indicative of intermediate formation in particular on the S2-S3 transition.

(2) Analysis of kinetic traces on the S2-S3 and S3-S0 (oxygen-evolving) transition (Fig. 2) allowed for the detection of deviations from mono-exponential behaviour. On the S3-S0 step, a lag-phase prior to Mn reduction by electrons from water was observed as previously. However, the lag duration in D₂O was longer,

~300 μ s, suggesting that it originates in intermediate formation coupled to a protolytic reaction. A similar, but considerably shorter lag phase of ~50-100 μ s became visible on the S2-S3 transition in D₂O (Fig. 3). This result provides new evidence that an intermediate is formed prior to Mn oxidation on this step, which also seems to be coupled to a protolytic reaction.

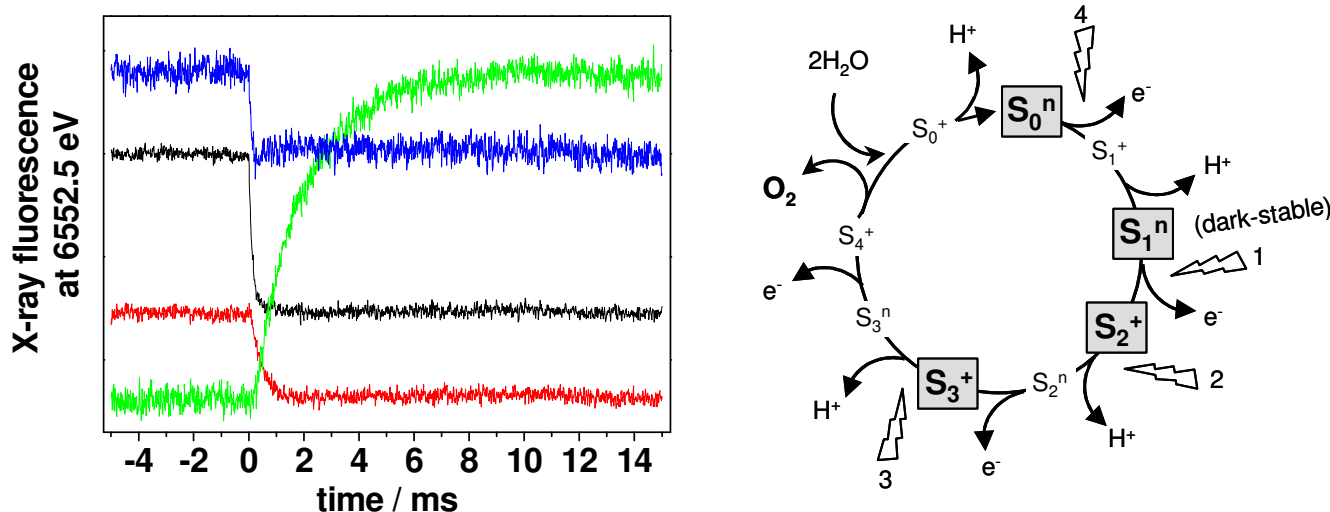


Fig. 1: (left) X-ray fluorescence transients on four S-transitions from PSII in D₂O. Decaying transients, Mn oxidation; uprising transient, Mn reduction. (right) Novel reaction cycle of water oxidation comprising intermediates on the S2-S3 (S₂ⁿ) and S3-S0 (S₃ⁿ) transitions [3].

Fig. 2: X-ray transient on the oxygen evolving S3-S0 transition (black) and simulation (red) showing increased duration of the lag-phase prior to Mn reduction. The arrow indicates the laser flash.

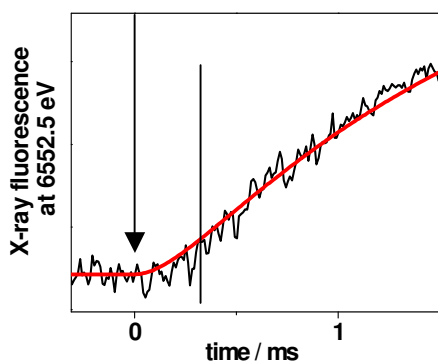
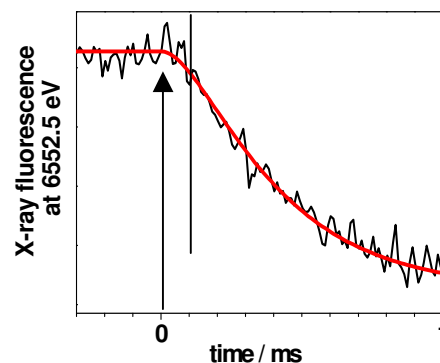


Fig. 3: X-ray transient on the S2-S3 transition (black) and simulation (red) showing lag-phase behaviour, which is indicative of intermediate formation prior to Mn oxidation.



(3) Timescan traces were recorded at 10 excitation energies in the region of the Mn K-edge. From these transients, decay-associated spectra at increasing times after the laser flash can be constructed. We are aiming at the detection of spectral features of the intermediates on S2-S3 and S3-S0; data evaluation is in progress.

(4) Mn K-edge spectra were recorded by rapid scans (1 s) after the application of 0-6 Laser flashes to the PSII samples in D₂O. Preliminary data evaluation suggests that there are no major spectral differences between K-edge spectra in D₂O and H₂O.

Conclusions:

We consider the run SC2478 as very successful. Time-resolved X-ray transients monitoring the reaction cycle of the PSII Mn complex with unprecedented quality were obtained. The data provide good evidence that there is an intermediate in the water oxidation cycle not only on the oxygen evolving S3-S0 transition, but also on the Mn oxidizing S2-S3 step. Apparently, both intermediates are coupled to protolytic reactions as their lifetimes were considerably increased in D₂O. These results may bring us one step closer to the complete unraveling of all intermediates in the intricate reaction cycle of water oxidation. A respective publication focusing on the partial reactions on the Mn oxidizing S2-S3 transition, which comprises the new time resolved data and also K-beta emission data collected during a previous run at ID26, is in preparation.

References:

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