


Experiment title:

 Characterization of the crucial S_4 state of photosynthetic O_2 evolution: Stabilization in time-resolved X-ray experiments by H/D-isotope and pH changes

Experiment number:

SC2258

Beamline:

ID26

Date of experiment:

from: 24.04.2007

to:

01.05.2007

Date of report:

27.08.2007

Shifts:

21

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Report: Water oxidizing photosynthesis producing dioxygen is a key reaction sustaining life on earth. In the future, light-driven water oxidation may be coupled to H_2 -production by hydrogenases for fuel production. O_2 formation proceeds at the manganese complex of photosystem II (PSII) in a complex reaction cycle involving a series of intermediates (S-states). We have established the existence of an intermediate, S_4 , in the crucial O_2 -evolving reaction step $S_3 \rightarrow S_0 + O_2$ [1-4]. (1) The properties of the S_4 state were scrutinized in time-resolved X-ray experiments at the Mn K-edge using laser flash excitation of PSII samples in the X-ray beam. The lifetime of the intermediate was extended by varying the sample pH and using the replacement of H_2O by D_2O . Most interestingly, the experiments provided evidence for a further intermediate in the Mn-oxidizing $S_2 \rightarrow S_3$ transition that needs to be further characterized. These observations strongly support our extended reaction cycle of photosynthetic water oxidation [2,4]. (2) Preliminary Resonant Inelastic X-ray Scattering (RIXS) experiments were performed on Ni-Fe hydrogenases and model compounds. RIXS on biological metal centers is feasible at ID26 and will be used in 2008 to study the Mn complex of PSII.

Results: Multilayer samples of PSII were prepared in H_2O at pH 6.3 and in D_2O at pD values of 5.8 and 6.8. The rationale was to kinetically label those reactions coupled to protolysis in the water oxidation cycle and to extend the lifetime of intermediates. Figure 1 shows X-ray transients in H_2O and D_2O on the four transitions between the semistable S-states. (1) The ratio of the rates k_H/k_D of Mn oxidation on transitions $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_1$ is close to one. These reactions apparently are not coupled to proton release. (2) There is a pronounced isotope effect on the $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_4 \rightarrow S_0$ transition where O_2 is produced. (3) The lag phase attributable to S_4 formation is prolonged by a factor of ~ 2 in D_2O . (4) In D_2O , there is evidence for a lag phase in the X-ray transient also in the $S_2 \rightarrow S_3$ transition which has not previously been observed (Fig. 2). This lag is the first experimental evidence for an intermediate also in this Mn oxidizing reaction step. (5) At a higher pD the rates of Mn oxidation/reduction and of intermediate formation were similar to those in H_2O , as expected for the compensation of H/D and pH effects.

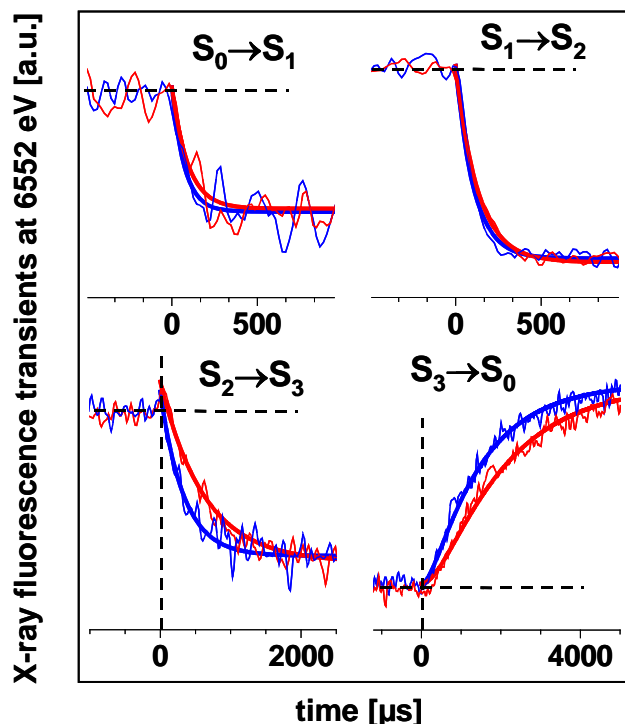


Fig. 1: Kinetic X-ray transients in H_2O (blue) and D_2O (red). Smooth lines = fit curves. About 1500 transients were averaged per trace; time resolution = 10 μs per data point.

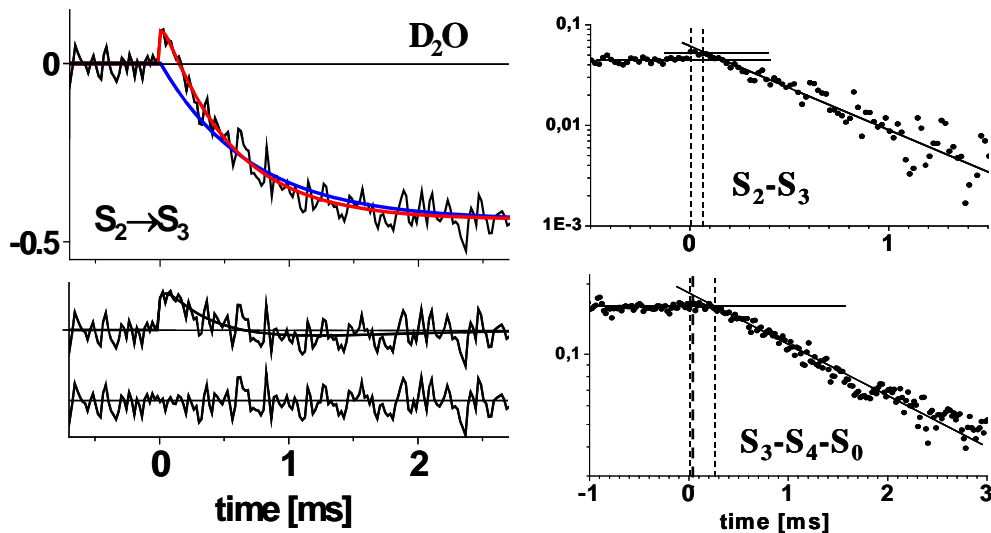
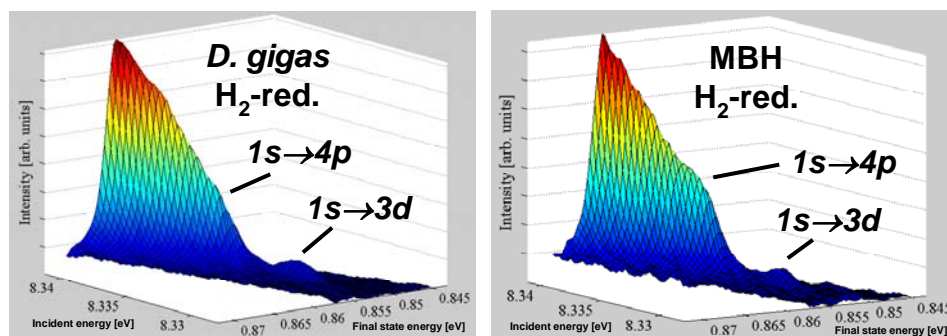


Fig. 2: Left: Kinetic X-ray transient on the $S_2 \rightarrow S_3$ transition; red = simulation using two consecutive reactions, blue = single exponential fit, bottom = residual plots. Right: Comparison of lag-phase behavior (dashes) on the O_2 -evolving step (bottom) and on $S_2 \rightarrow S_3$ (top).

The H/D and pH effects reveal: (1) The formation of the S_4 state is coupled to a deprotonation at the Mn complex and not to Mn oxidation. This deprotonation may be crucial for subsequent O_2 formation [**]. (2) The first observation of a lag in the $S_2 \rightarrow S_3$ transition, particularly in D_2O , may point to a crucial deprotonation reaction also in this step. It is highly important to characterize the nature of this state in further time-resolved X-ray experiments. The preliminary evidence for an intermediate in the $S_2 \rightarrow S_3$ step may bring us closer to the characterization of all of the at least eight states [2] in the reaction cycle of water oxidation.

Preliminary RIXS experiments at the Ni K-edge were performed on 5 different Ni-Fe hydrogenases and on model compounds (obtained from Profs. B. Friedrich, M. Driess, C. Limberg (all Berlin) and A. DeLacey, Madrid) on the single-crystal spectrometer at ID26 (Fig. 3). We are grateful for additional in-house beamtime. RIXS on ultra-dilute (1 mM metal) biological samples is feasible. Radiation damage can be minimized; the signal-to-noise ratio is reasonable. Mn RIXS measurements are planned in February 2008.

Fig. 3: RIXS planes at the Ni $K_{\alpha 1}$ emission line for two Ni-Fe hydrogenase samples (left, *Desulfovibrio gigas*; right, membrane-bound hydrogenase of *Ralstonia eutropha*). Ten RIXS scans of ~10 min duration were averaged. Proteins were in the hydrogen-reduced state.



Conclusions: We consider the April run as particularly successful. Valuable new information on intermediates in the O_2 -evolving reaction cycle of water oxidation has been obtained which supports our hypothesis of the involvement of crucial deprotonation reactions at the Mn complex [1-4]. The results on the O_2 -evolving step are part of a manuscript in preparation. For the first time, preliminary evidence for a further intermediate in the $S_2 \rightarrow S_3$ transition became visible. It is highly important to characterize this intermediate in future time-resolved X-ray experiments.

Preliminary RIXS data have been collected at the Ni K-edge and the feasibility of RIXS on biological samples at ID26 was established. These challenging experiments are expected to provide high-resolution information on the atomic structure and also on the electronic configuration of metal sites in the future.

References

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- [4] H. Dau, M. Haumann, *Coord. Chem. Rev.*, accepted for publication (2007).