


**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:**

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01.05.2007

**Date of report:**

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**Shifts:**

21

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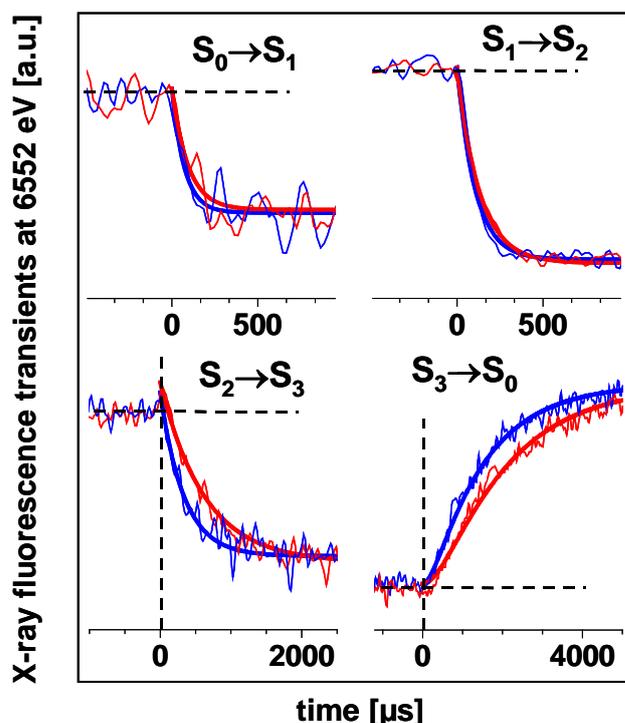
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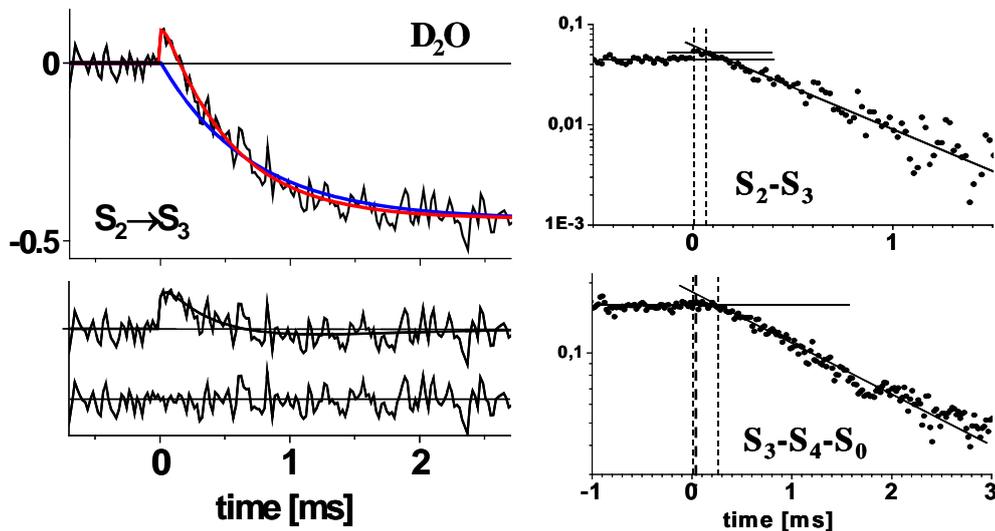
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**Report:** Water oxidizing photosynthesis producing dioxygen is a key reaction sustaining life on earth. In the future, light-driven water oxidation may be coupled to  $H_2$ -production by hydrogenases for fuel production.  $O_2$  formation proceeds 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  $\sim 2$  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  $\mu s$  per data point.

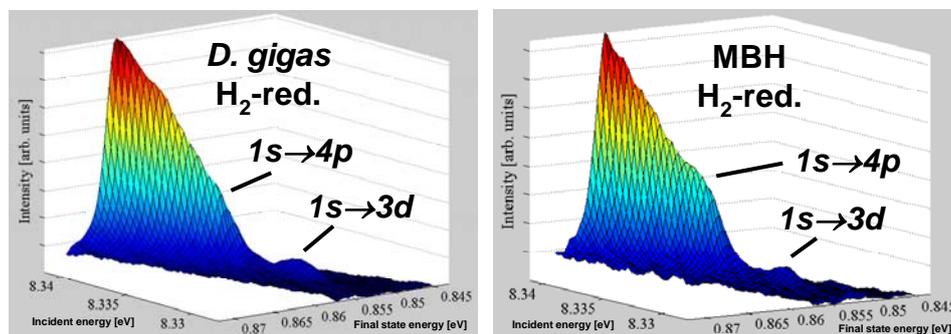


**Fig. 2:** Left: Kinetic X-ray transient on the  $S_2 \rightarrow S_3$  transition; red = simulation using two consecutive reactions, blue = single exponential fit, bottom = residual plots. Right: Comparison of lag-phase behavior (dashes) on the  $O_2$ -evolving step (bottom) and on  $S_2 \rightarrow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