



	Experiment title: Short-lived intermediates in O ₂ formation by photosystem II studied by time-resolved X-ray absorption spectroscopy	Experiment number: SC1909
Beamline: ID26	Date of experiment: from: 23.11.2005 to: 29.11.2005	Date of report: 15.02.2006
Shifts: 18	Local contact(s): Dr. Olga Safonova	<i>Received at ESRF:</i>

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Report: Photosynthetic water oxidation produces the oxygen of the atmosphere. O₂ formation is catalyzed by a Mn₄Ca complex bound to photosystem II (PSII) and electron transfer (ET) involves two plastoquinones and a non-heme iron located in between. Recently, we have detected, for the first time, the enigmatic S₄ state of the catalytic cycle of the Mn complex by a time-resolved X-ray experiment [1]. In the present study we performed three different experiments: **(A)** The S₄ state is apparent by a lag-phase (~150 μs) in Mn reduction by electrons from water as detected by X-ray fluorescence changes [1-3]; S₄ formation is attributed to deprotonation of the Mn complex [1]. We studied the effect of H₂O/D₂O exchange on the lag-phase and on the rates of ET from Mn to the tyrosine_Z by time-resolved XAS at the Mn K-edge to gain more information on S₄. **(B)** A further crucial intermediate of the O₂-evolving step has been detected by monitoring ET at the Mn complex at elevated "O₂ backpressure" thereby stabilizing a putatively partially reduced state S₂* [4]. We measured first Mn XANES spectra of four S-states at 10-15 bar O₂ pressure in a newly constructed pressure cell suitable for XAS. **(C)** The non-heme iron may be crucially involved in ET between the quinones [5,6]. By time-resolved XAS (5 μs) at the Fe K-edge we investigated its reactions in PSII after Laser flash excitation.

Experimental: Highly active PSII samples for XAS were prepared as in [1,2] (total of about 6000 samples). X-ray fluorescence measurements at the Mn and Fe K-edge were performed at room temperature with our scintillation detector. Signals were recorded on a PC (20 MHz A/D card) by a home-made transient-recorder program and simultaneously by the beamline electronics. Flash-and-rapid-scan XANES spectra were collected by simultaneous scanning of the Si220 crystal monochromator and the undulator gap within ~1 s. The energy axis was calibrated using a KMnO₄ standard or an Fe foil. For details on the time-resolved XAS techniques see [1-3]. Samples were illuminated in the X-ray beam by Laser flashes (recently purchased Q-switched, frequency-doubled Nd-YAG Laser, Continuum Inlite-II, λ = 532 nm, beam diameter ~6 mm, 150 mJ per 5 ns pulse). Between the flashes, the X-ray beam was blocked by a rapid photoshutter, which opened only for ±20 ms around the flash, to minimize X-ray photoreduction. Samples were positioned in the X-ray beam by a computer-controlled sample-changer. To maximize the X-ray flux and thereby the signal-to-noise ratio of kinetic traces, the three undulators of ID26 were employed simultaneously.

Results:

(A) Mn X-ray fluorescence changes were monitored in the rising part of the K-edge during the first four laser flashes in H₂O and D₂O (Fig. 1). Kinetic analysis of the transients indicated that the effect on the electron transfer rate (k_{H/D}) from Mn to Tyr was largest on transitions S₂→S₃ and S₃→S₄ of the catalytic cycle of water oxidation. These results strengthen our previous notion that these steps, and particularly S₄ formation [1], are kinetically steered by proton release. Our next step will be the characterization of S₄ by EXAFS analysis in microseconds.

(B) A newly constructed pressure cell housing 20 PSII samples and sustaining pO₂ up to 20 bar for the first time was employed for studies of the Mn complex at 10-15 bar O₂. The cell was positioned in the beam by the computer controlled linear stage for rapid sample exchange. Figure 2 shows XANES spectra obtained at ambient and 10-15 bar O₂ pressure after the Laser flashes given ~200 ms prior to the start of the scan. The spectra reveal that at both pressures there is Mn oxidation on flashes 1 and 2. Mn reduction occurs on the O₂ evolving step (3rd flash) also at high pO₂. However, there are uncertainties with respect to X-ray photoreduction of Mn [7] (less at high pO₂, not shown) and saturation of samples with O₂. Therefore, quantitative comparison of the edge shifts on the flashes is not possible. We will construct an improved pressure cell and apply for a measuring period to proceed with the highly interesting pressure experiments.

(C) In PSII samples where the non-heme Fe was preoxidized by ferricyanide, for the first time its reduction was followed directly by XAS at 7126 eV. Fe reduction by reduced quinone_A (Q_A⁻) on flash 1 occurred with $t_{1/2} \sim 15 \mu\text{s}$ (Fig. 3A). Even more interesting were changes on flash 2 where ET Q_A⁻ → Q_B occurs. The respective changes (Fig. 3B) are interpreted as indicating the formation, from 6-coordinated Fe, in $\sim 100 \mu\text{s}$ and decay in $\sim 1.5 \text{ ms}$ of 5-coordinated Fe which binds bicarbonate [8] in a monodentate mode. This coordination change may facilitate efficient ET. A manuscript on these exciting results is in preparation.

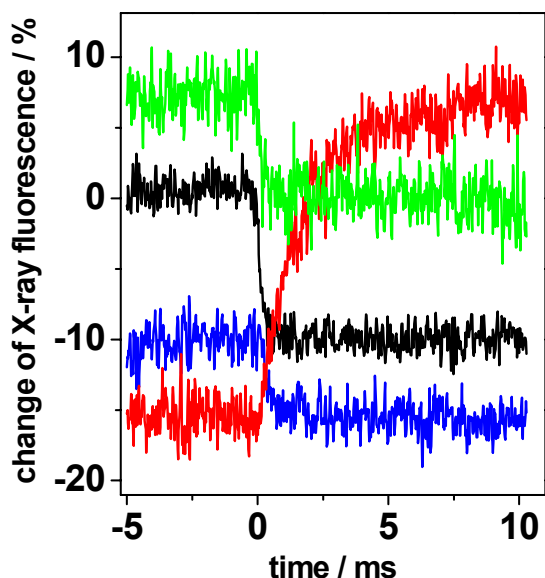


Fig. 1: Mn X-ray fluorescence transients (10 μs per data point) at an excitation energy of 6552 eV on laser flashes (at $t = 0$) 1 (black), 2 (blue), 3 (red), 4 (green) from PSII particles in D₂O (deconvoluted for miss contributions) reflecting ET Mn → Tyr_Z⁺. 600 transients from fresh PSII samples were averaged.

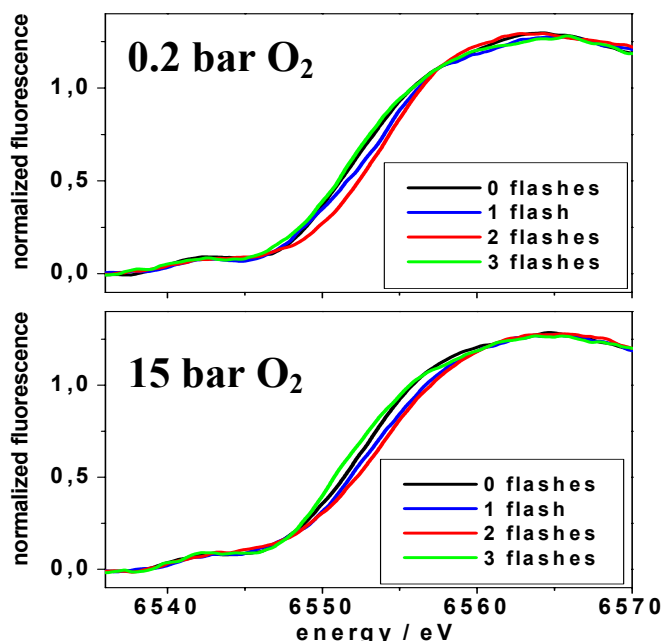


Fig. 2: Flash-and-rapid-scan XANES spectra of the Mn complex in 4 S-states at ambient and high pO₂ all measured with PSII samples in a novel pressure cell (~ 60 scans per spectrum). Note the change in edge position between red (S₃) and green (S₀ or S₂^{*}) spectra.

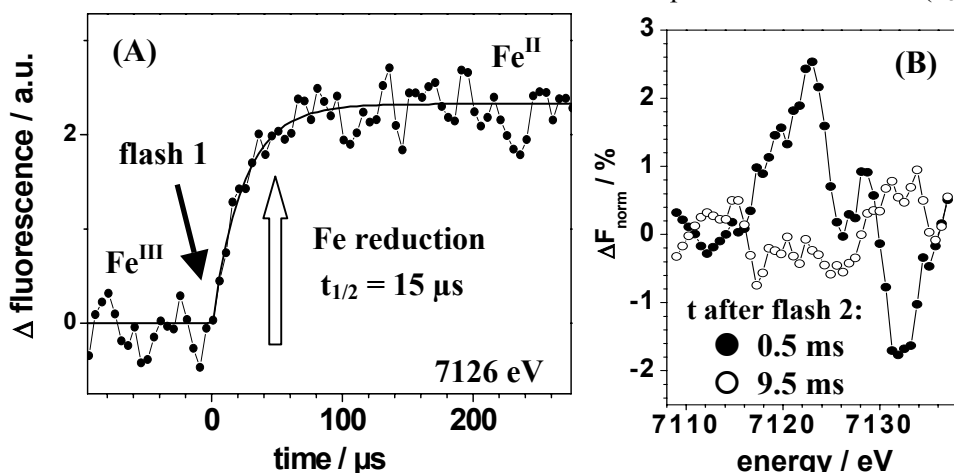


Fig. 3: (A) Reduction of the non-heme Fe of PSII by Q_A⁻ monitored by fluorescence changes (5 μs per point, 1000 transients averaged). (B) Decay-associated spectra of changes of the Fe K-edge after flash 2. The derivative-shaped spectrum at 0.5 ms reflects a transient coordination change (of bicarbonate) at the Fe.

Summary: We consider the November run as particularly successful. Three different time-resolved XAS experiments at the Mn and Fe K-edge have been performed and novel information on highly debated questions on photosynthetic water oxidation has been obtained. Successful testing of a pressure cell opens the road for XAS on proteins at elevated gas pressure. We will extend these experiments in the next period.

We thank the scientists at ID26 (Drs. O. Safonova, S. Eeckhout, and P. Glatzel) for outstanding engagement and the Deutsche Forschungsgemeinschaft (SFB498, projects C6 and C8) for financial support.

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