



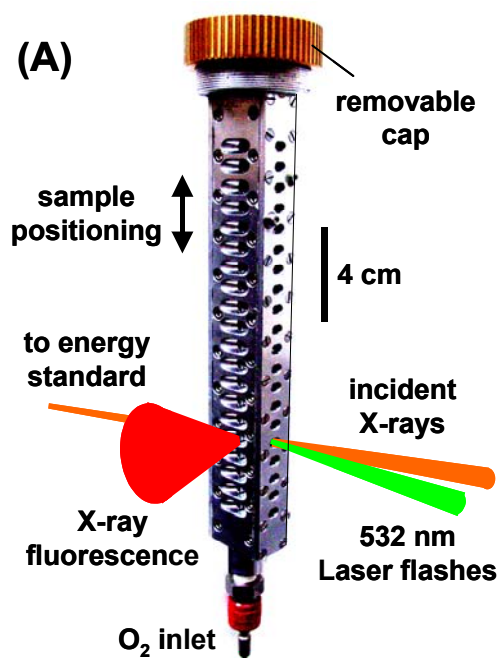
	<b>Experiment title:</b> Intermediates in photosynthetic oxygen evolution stabilized at elevated O <sub>2</sub> “backpressure” and analyzed by flash-and-rapid-scan EXAFS and time-resolved X-ray experiments at the Mn K-edge	<b>Experiment number:</b> SC2140
<b>Beamline:</b> ID26	<b>Date of experiment:</b> from: 06.12.2006 to: 18.12.2006	<b>Date of report:</b> 17.04.2007
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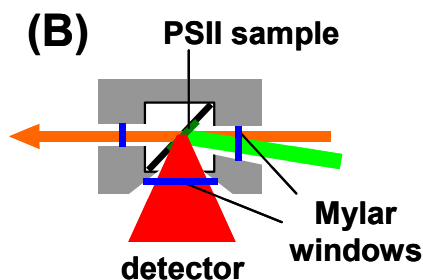
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**Report:** Photosynthetic water oxidation is a key reaction in Nature that sustains life on Earth. Dioxygen is produced at a Mn<sub>4</sub>Ca complex bound to photosystem II (PSII) [1]. Recent progress in the understanding of water oxidation comes from the characterization of novel intermediates in the O<sub>2</sub>-evolving step of the catalytic cycle of the Mn complex as stabilized at elevated product “backpressure”, i.e. at 10-30 bar O<sub>2</sub> [2,3] and of the S<sub>4</sub> state by time-resolved X-ray experiments [4]. Around 10 bar O<sub>2</sub>, the reduction of the previously three-fold oxidized Mn complex by electrons from water seems to be incomplete on the O<sub>2</sub>-evolving transition [2,3] giving rise to an intermediate which may also be crucially involved at ambient pO<sub>2</sub>. We studied the Mn complex in its catalytic cycle by time-resolved X-ray absorption spectroscopy (XAS) at the Mn K-edge, at room temperature, and at elevated O<sub>2</sub> pressure at beamline ID26. Previously established kinetic X-ray techniques [4,5] were combined with a newly constructed pressure cell, allowing for XAS on protein samples at gas pressures ≤20 bar. The data reveal various effects of elevated pO<sub>2</sub> on PSII; an intermediate in the O<sub>2</sub>-evolving step is not uniquely implied by, but likely compatible with the data.

**Experimental:** A pressure cell for X-ray experiments on PSII protein has been constructed in our laboratory (Fig. 1). It allows for XAS experiments at up to 20 bar O<sub>2</sub> and for illumination of the 20 samples in the cell by ns-Laser flashes during the X-ray measurements. Extensive testing of the cell revealed that the signal-to-noise ratio in kinetic X-ray experiments is sufficient to obtain a time-resolution of 10 μs. XANES spectra (1 s duration) and timescan fluorescence traces (10 μs resolution) were recorded on PSII in the cell after Laser flash illumination.



**Fig. 1:** (A) Photograph of the O<sub>2</sub> pressure cell which was mounted on a linear stage and beam alignment. The X-ray fluorescence was monitored by a scintillation detector. (B) Irradiation geometry of the PSII samples.



**Results: (1)** The rate of X-ray photoreduction of the Mn complex [6] extensively was studied as function of the O<sub>2</sub> pressure (data not shown). The data suggested that there was no specific effect of the pO<sub>2</sub> on the reduction rate. However, due to the increased X-ray absorbance by the gas at elevated pO<sub>2</sub>, comparison of X-ray data at ambient and high pO<sub>2</sub> required the adjustment of the X-ray intensity on the samples to the same level under both conditions, which was achieved using the Al attenuator box of the ID26 beamline.

(2) XANES spectra of the Mn complex after zero to six Laser flashes applied immediately before the start of data collection were recorded at ambient  $pO_2$  and at 10 bar  $O_2$  (Fig. 2A). The good-quality spectra revealed that the pattern of the edge energy as function of the flash number differed at 10 bar (Fig. 2B). Data analysis suggested partial oxidation of the Mn complex by 10 bar  $O_2$  in the dark. The 3<sup>rd</sup> Laser flash induces the  $O_2$ -producing reaction. The XANES spectrum after this flash at ambient  $pO_2$  ( $S_0$  state) differs from that at 10 bar, pointing to the occurrence of an intermediate in the  $O_2$ -evolving step in a fraction of PSII centers.

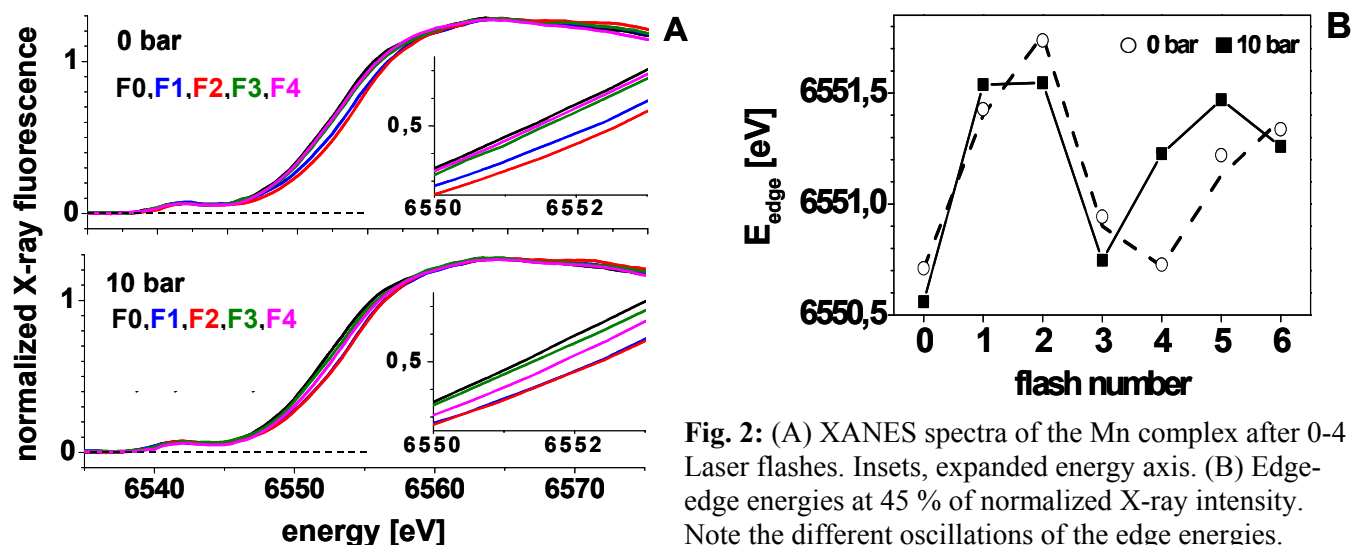


Fig. 2: (A) XANES spectra of the Mn complex after 0-4 Laser flashes. Insets, expanded energy axis. (B) Edge-energy energies at 45 % of normalized X-ray intensity. Note the different oscillations of the edge energies.

(3) Timescan fluorescence traces at an excitation energy of 6552 eV were recorded to monitor the stepping of the Mn complex through its catalytic cycle (Fig. 3A). At 0.2 bar and 12 bar  $O_2$ , there was an oscillatory behaviour of the fluorescence level, suggesting that  $O_2$  formation occurred to a significant extent also at 12 bar  $O_2$ . The different oscillatory behaviour (Fig. 3B,C), however, pointed to an altered S-state distribution in the dark and was compatible with intermediate formation in a fraction of the PSII centers at 12 bar  $O_2$ .

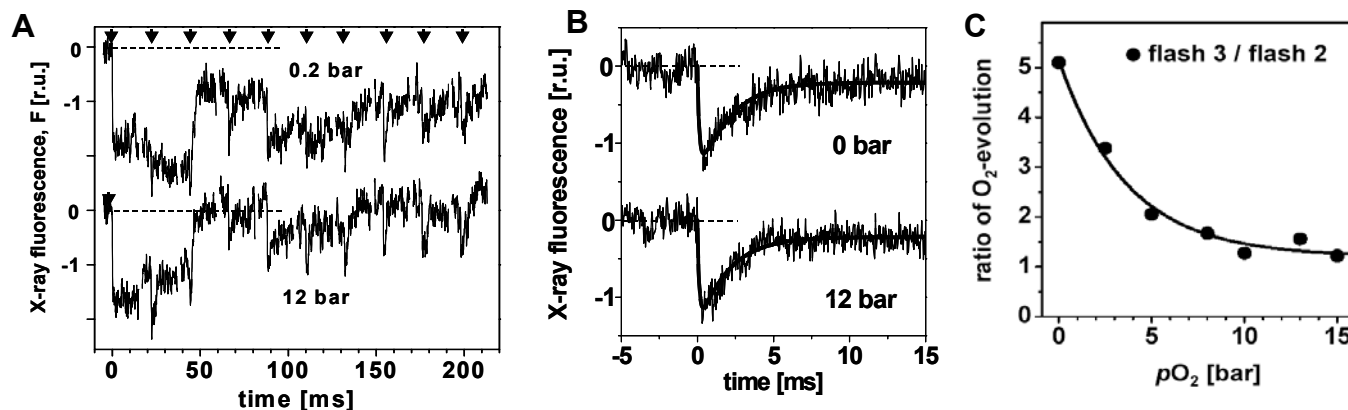


Fig. 3: (A) Timescan traces at 6552 eV (arrows = flashes). (B) The average of timescan traces on flashes 1 to 10 shows  $O_2$ -formation in  $\sim 1$  ms also at 12 bar. (C) Increased  $O_2$  evolution on flash 2 at high  $pO_2$ .

**Summary:** The catalytic cycle of the Mn complex of PSII [7] successfully was studied by X-ray spectroscopy at ID26 at high  $pO_2$  in a new pressure cell. XANES spectra of high quality were obtained. Timescan traces at 10  $\mu s$  resolution allowed for estimation of the rates of  $O_2$ -formation and of the lower S-transitions. The XAS data are compatible with formation of an intermediate at elevated  $pO_2$ . Current in-depth data analysis may provide further characterization of this state. A respective publication is in preparation. In summary, we have performed a successful attempt to established time-resolved X-ray experiments at elevated gas pressure under Laser flash excitation to study metal centers of proteins in their catalytic cycle.

#### References:

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